MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 124 APRIL–JUNE 2013

Chaetomium jatrophae sp. nov.
(Sharma & al. — PLATE 2, p. 122)
ROHIT SHARMA, *artist*

ISSN (PRINT) 0093-4666 http://dx.doi.org/10.5248/124 ISSN (ONLINE) 2154-8889
MYXNAE 124: 1–368 (2013)

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Published by
Mycotaxon, Ltd.
p.o. box 264, Ithaca, NY 14581-0264, USA
www.mycotaxon.com & www.ingentaconnect.com/content/mtax/mt
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MYCOTAXON

THE INTERNATIONAL JOURNAL OF FUNGAL TAXONOMY & NOMENCLATURE

VOLUME 124

APRIL–JUNE, 2013

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CONSISTING OF I–XII + 368 PAGES INCLUDING FIGURES

ISSN 0093-4666 (PRINT) http://dx.doi.org/10.5248/124.cvr ISSN 2154-8889 (ONLINE)

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MYCOTAXON

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p.30, table, after *Lambertella* sp. 3 FOR: Leaf of *Pinus* sp. READ: Leaf of *Pyrola* sp.
p.30, table, after *Lambertella* sp. 8 FOR: Leaf of *Pinus* sp. READ: Leaf of *Pyrola* sp.

Volume 123

p. ii, lines 12–13
FOR: Seppo Huhtinen (2006–2012) *Turku, Finland*
READ: Karen Hansen (2013–2017) *Stockholm, Sweden*

p. iv, line 12:
FOR: *Lichenochora tertia*
READ: *Lichenochora tertia* (*Phyllochorales*):

p. iv, line 22:
FOR: *Jatropha podarica* READ: *Jatropha podagrica*

p. vi, line 8:
FOR: Teodor T. Denchev, Habib Ahmad & Abdul Nasir Khalid
READ: Teodor T. Denchev, Abdul Nasir Khalid & Habib Ahmad

publication date for volume one hundred twenty-three
MYCOTAXON *for* January–March, volume 123 (i–vi + 1–495)
was issued on August 1, 2013

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The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this volume.

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From the Editor-in-Chief

Welcome Karen Hansen! — We wish to announce – somewhat belatedly – that Sweden's Karen Hansen (Senior Curator, Kryptogambotanik, Naturhistoriska Riksmuseet, Stockholm) has been elected to Mycotaxon's Editorial Advisory Board. She joins Chair Wen-Ying Zhuang (Beijing), Past Chair Henning Knudsen (Copenhagen), Scott Redhead (Ottawa), Sabine Huhndorf (Chicago), and Peter Buchanan (Auckland) on the 2013 Board. Each member serves a six-year term, standing as Chair in the fifth year and as Past Chair in his or her final year. We apologize profusely for the editorial (!) lapse leading to the omission of Karen's name from the masthead (on p. ii) of Mycotaxon 123, the first volume for 2013.

Mycotaxon 124 — After some deliberation and in an effort to bring the journal back on schedule this year, we have closed the delayed April–June 2013 volume at 380 pages. The current volume contains 38 papers by 151 authors (representing 26 countries) and revised by 74 expert reviewers.

Within its pages are one new genus and species (*Scolecobeltrania* from Venezuela) and 33 other taxa new to science representing *Arenariomyces* from Denmark; *Beauveria, Entoloma, Glomus, Inonotus, Kuklospora, Myrmecridium, Pertusaria, Phialophora, Phylloporia, Russula, Tuber,* and *Xerocomus* from China; *Chaetomium* from India; *Cladonia* and *Septoglomus* from Brazil; *Coleosporium* from Europe; *Hypochnicium* from East Asia; *Leccinellum* from the U.S.A.; *Lyromma* from Bolivia and Brazil; *Pisolithus* and *Terfezia* from Portugal and/or Spain; and *Polycoccum* from Turkey.

In addition to range extensions and/or new hosts for previously named taxa, we also offer new combinations in *Aspicilia, Cantharocybe, Lactarius,* and *Rutstroemia,* newly established synonymies and typifications, and conclusions from recent mycorrhizal (*Inocybe*) and monographic (*Fissurina* in Vietnam, plectomycetes in Italy, ascomycetes in Guatemala) studies.

The next two volumes will also close 'early' to ensure that the four 2013 volumes will not spill over into next year. We shall begin 2014 with a much shorter turn-around time between final submission and publication, and hope eventually to return to publishing each volume at the beginning of a quarter rather than well after!

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
29 September 2013

ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.1

Volume 124, pp. 1–8 *April–June 2013*

A new *Myrmecridium* species from Guizhou, China

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Abstract — A strain of *Myrmecridium* was isolated from farmland soil in Guizhou Province, China. The strain produced distinctive obovoid or fusoid conidia that taper to a subtruncate darkened hilum at the base that distinguished it from other *Myrmecridium* species. Phylogenetic analysis of combined ITS1–5.8S–ITS2 and large subunit rDNA sequences supported this morphological separation. We describe *Myrmecridium obovoideum* as new and provide a key to *Myrmecridium* species.

Key words — asexual fungi, hyphomycetes, molecular phylogeny

Introduction

Arzanlou et al. (2007) established *Myrmecridium* Arzanlou et al. based on its hyaline mycelium with pale to unpigmented, pimple-like denticles that produce obovoid or fusiform conidia, tapering towards a narrowly truncate base with a slightly prominent unpigmented hilum and schizolytic conidial secession. The genus belongs in *Sordariomycetes* and comprises three species and two varieties (Arzanlou et al. 2007; Crous et al. 2011; Crous et al. 2012).

During a survey of anamorphic ascomycetes, we isolated a strain with characters of *Myrmecridium* (Arzanlou et al. 2007) from farmland soil in China.

[A] C.-Y. Jie and Q.-X. Zhou contributed equally to the manuscript and should be considered as joint first authors

Combined ITS and LSU rDNA sequences were generated from the strain and analyzed to evaluate the morphological findings. Based on morphology and DNA sequence comparison, the strain is proposed as *Myrmecridium obovoideum*. In the present paper, we provide detailed illustrations and a description of this new species.

Materials & methods

Morphological and cultural studies

The *Myrmecridium* specimen — from a farmland soil sample collected from Huaxi District, Guizhou, China, in 2005 —is deposited in the herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP), and ex-type strains are conserved in HSAUP and the Herbarium of Plant Pathology, Guizhou University (HGUP). The specimen was examined in the laboratory and the taxon is described from cultures grown at 25°C on potato dextrose agar (PDA). Conidia and conidiophores were placed in a drop of 85% lactic acid and examined and photographed using a Nikon 80i microscope (Nikon Corporation, Japan) at 400× and 1000× magnification. The taxonomic determinations were made by comparing with descriptions in the literature.

DNA extraction, amplification and DNA sequencing

Genomic DNA was extracted from colonies grown on potato-dextrose agar (PDA), using the Fungal gDNA Kit GD2416 (Biomiga, CA, USA) following the manufacturer's instructions. The universal primers ITS1/ITS4 (White et al. 1990) were used for the ITS region (ITS1–5.8S–ITS2) amplification, and LR0R/LR5 were used for a segment of the large subunit rDNA (Vilgalys and Hester 1990). Amplification reactions were performed in a Bio-RAD PTC-200 thermocycler in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 U of Taq polymerase.

The PCR-amplified DNA fragments were fractionated in 1% agarose gels in 0.5× TBE buffer and visualized by ethidium bromide staining and UV illumination. Sequencing was performed with an ABI PRISM 3730 DNA autosequencer using either dRhodamine terminator or Big Dye Terminator chemistry (Life Technologies™, USA). Sequence data of the isolates used in the study were deposited in GenBank (Table 1). Alignments are available in TreeBASE (www.treebase.org/treebase-web/home.html) under the study ID 13733.

Phylogenetic analyses

Preliminary nucleotide sequence alignments were constructed using Clustal X 1.81 (Thompson et al. 1997). A partition homogeneity test (Farris et al. 1994) was applied to evaluate the feasibility of combining the datasets. Phylogenetic analyses of LSU sequences and combined ITS and LSU rDNA sequence were computed using MP analysis in PAUP* (Swofford 2002). In the MP analyses, trees were inferred using heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions, maxtrees were 5000, branches of zero length were collapsed and all parsimonious trees were saved. Measures calculated for parsimony

included tree length (TL), consistency index (CI), retention index (RI), and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were conducted with 1000 replications.

Results

Phylogenetic analysis

Partition homogeneity tests for combining the two (ITS and LSU) gene regions yielded a P-value of 0.104, and the two gene/regions were combined based on the tree topologies and P-value (Cunningham 1997, Dettman et

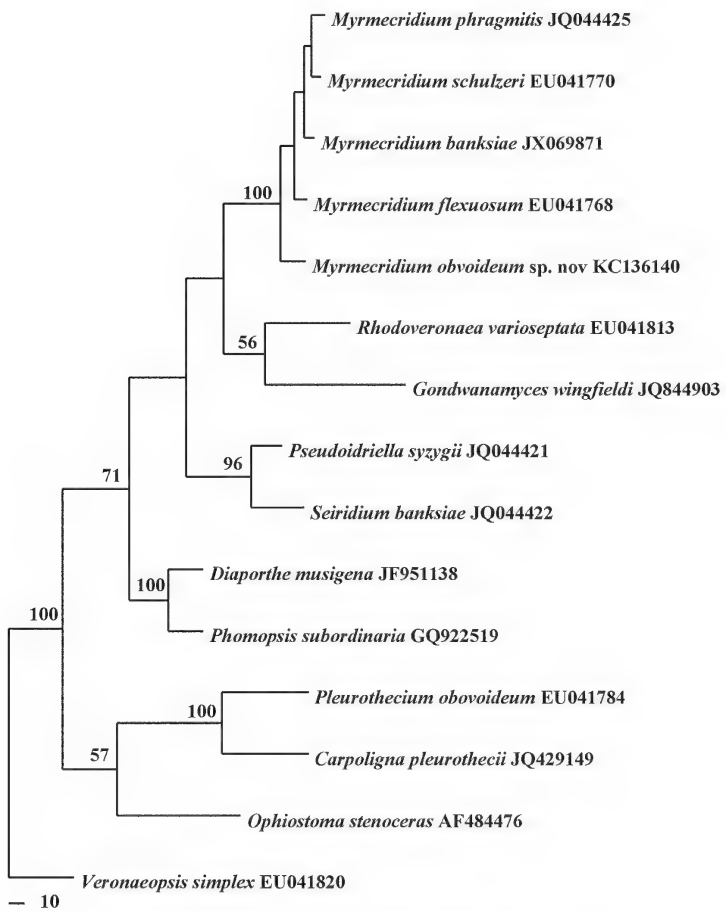

FIG. 1. One of the two equally most parsimonious trees of the analyzed ITS and LSU rDNA region/genes (284 of 1485 characters were parsimony informative). Bootstrap support values of less than 50% are not shown. The tree is rooted with *Pleurothecium obovoideum*.

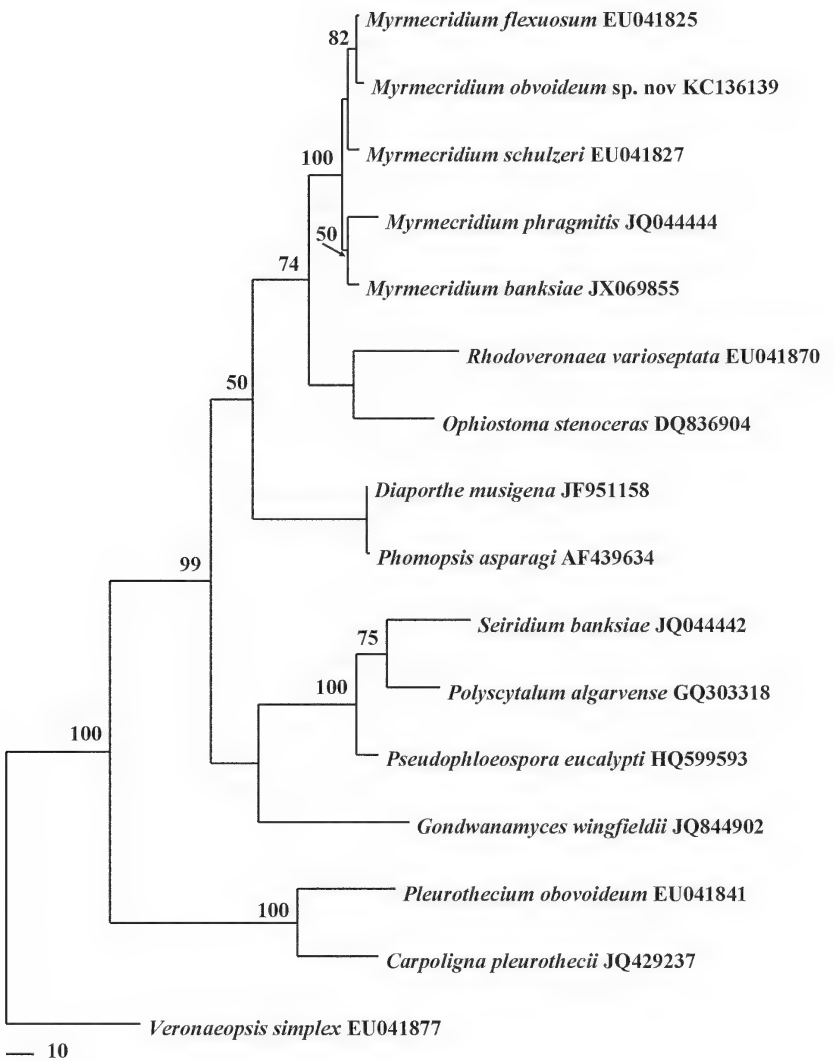

FIG. 2. The most parsimonious tree of the analyzed LSU rDNA gene (206 of 844 characters were parsimony informative). Bootstrap support values of less than 50% are not shown. The tree is rooted with *Veronaeopsis simplex*.

al. 2003). The aligned sequence data matrix contained five taxa (including the outgroup *Pleurothecium obovoideum*) and 1485 (546 ITS and 839 LSU) characters, of which 284 were parsimony informative. Two most parsimonious trees were obtained, with one chosen to represent the topology of the strict

consensus tree (Fig. 1; Tree Length (TL) = 380, CI = 0.956, RI = 0.467, HI = 0.044, and RC = 0.446). In this tree, five *Myrmecridium* species clustered together supported by a 100% bootstrap value. *Myrmecridium obovoideum* formed a single branch as the sister clade to the other four species with only 56% bootstrap support.

In order to evaluate the relationship of *Myrmecridium* with other ascomycetes, we also generated a parsimonious tree (Fig. 2) based on LSU rDNA gene region. The alignment with 16 taxa (*Veronaeopsis simplex* as outgroup) comprised 844 characters, of which 206 were parsimony-informative [Tree Length (TL) = 628, CI = 0.67, RI = 0.68, HI = 0.33, and RC = 0.45]. Only one most parsimonious tree was obtained to represent the topology of the strict consensus tree selected for presentation (Fig. 2). In this tree, the *Myrmecridium* group had a close relationship with *Rhodoveronaea* and *Ophiostoma* supported by a credible bootstrap value (74%), which was consistent with Arzanlou et al. (2007). However, the connection between *Rhodoveronaea* and *Ophiostoma* received weak support (<50%). Meanwhile, the placement of *M. obovoideum* was movable, because it showed a closer relationship with *M. flexuosum* (de Hoog) Arzanlou et al. supported by 82% bootstrap value, which differed from the analysis combined ITS and LSU rDNA gene regions.

Taxonomy

Myrmecridium obovoideum Jie, Y.L. Jiang, McKenzie & Yong Wang bis, **sp. nov.** Fig. 3

MycoBank MB 804082

Differs from other *Myrmecridium* species by its conidia with darkened hilum.

Type: China, Guizhou Province, Guiyang, Huaxi District, isolated from farmland soil, 5 November 2005, Y.L. Jiang (**Holotype**, HSAUP051001; **isotype**, HGUP0314; GenBank, KC136140, KC136139).

Etymology: in reference to the conidial shape of this new taxon.

Colonies on PDA at 25°C for 2 weeks reaching 30 mm diam. The surface effuse, grayish. Mycelium mostly superficial, partly immersed is contradictory with lacking aerial mycelium. Conidiophores straight to geniculate-sinuous, unbranched, subhyaline, 1–8-septate, ≤280 μm tall, 3–3.6 μm wide. Conidiogenous cells terminal, integrated, cylindrical, pale or reddish brown near the base, gradually becoming paler towards the apex, 50–210 μm, often with 1–6 thin septa, forming a rachis with scattered pimple-shaped denticles less than 1 μm long, sometimes fertile part slightly inflated. Conidia solitary, aseptate, subhyaline, pale brown, thin-walled, smooth to finely verrucose, surrounded by a wing-like gelatinous sheath, ≤1.5 μm thick, obovoid or fusoid, (7–)8–9(–10) × (3–)3.5(–4.2) μm, tapering to a subtruncate, slightly thickened hilum; darkened at rounded end.

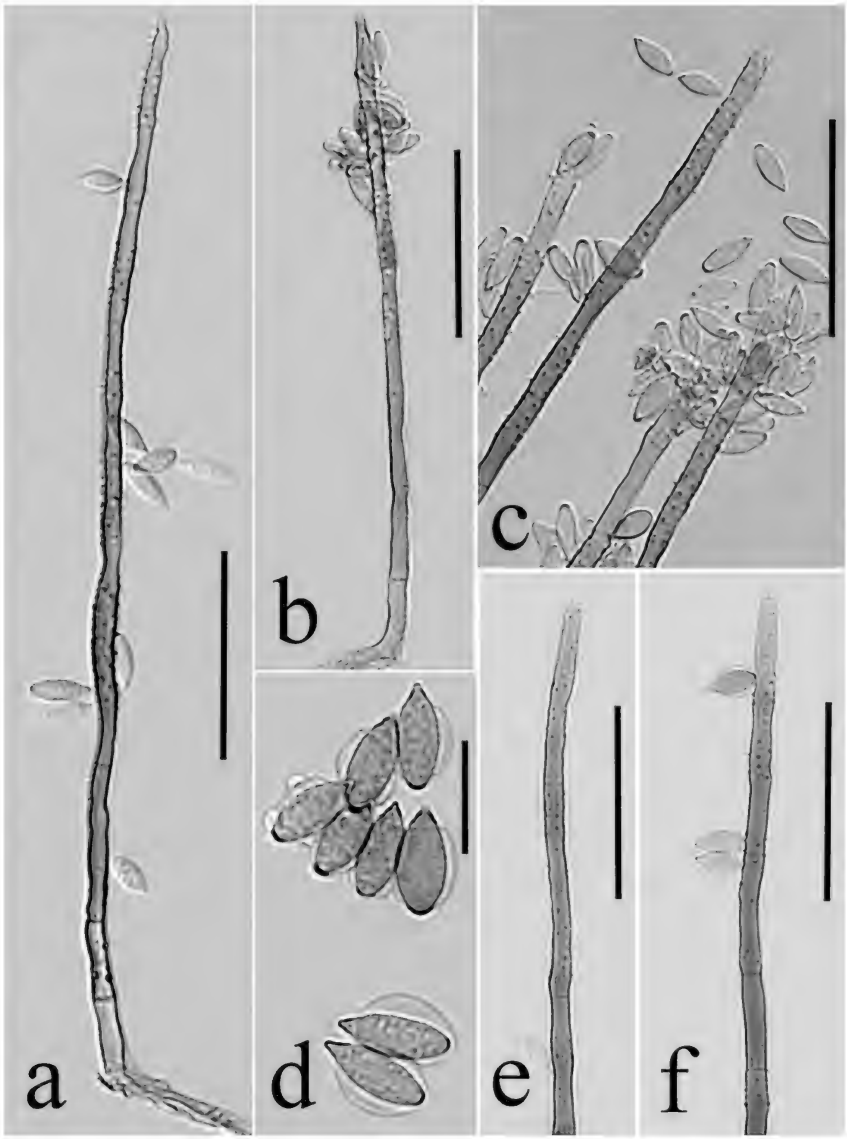

Fig. 3. *Myrmecridium obovoideum* (HSAUP051001, holotype) on PDA. a–b. Conidiophores. c. Rachis with pimple-shaped denticles and slightly inflated fertile part. d. Conidia surrounded by wing-like gelatinous sheaths. e–f. The thin septa of conidiogenous cells. Scale bars: a–c, e–f = 35 μm; d = 10 μm.

Discussion

Our LSU rDNA sequence analysis clustered *Myrmecridium* with *Rhodoveronaea* (sexual state = *Annulatascaceae*) and *Ophiostoma* (*Ophiostomataceae*) in the *Sordariomycetes*. Arzanlou et al. (2007) classified *Myrmecridium* as "*Incertae sedis* (*Sordariomycetes*)", and our results confirm this placement, without any indication of a family affinity.

Only *M. schulzeri* var. *tritici* (M.B. Ellis) Arzanlou et al. and *M. phragmitis* Crous produce septate conidia. *Myrmecridium banksiae* Crous differs from *M. obovoideum* by its larger conidia (9–14 × 2.5–3.5 µm); and *M. flexuosum* differs by its smaller conidia (5–9 × 3–4 µm). The conidia of *M. schulzeri* (Sacc.) Arzanlou et al. var. *schulzeri* (6–12 × 3–4 µm) are also larger than those of our species. In *Myrmecridium* only *M. obovoideum* has conidia with a darkened hilum, which enlarges the taxonomic criteria of *Myrmecridium* (Arzanlou et al. 2007). Our ITS and LSU rDNA sequence analyses also confirmed with high bootstrap value that our species differs from other *Myrmecridium* species. The combined morphological and phylogenetic analysis supports *M. obovoideum* as a new species.

Key to *Myrmecridium* species

1a. Hilum of conidia not darkened 2
1b. Hilum of conidia darkened ..... *M. obovoideum*

2a. Conidia without septate 3
2b. Septate conidia frequently observed ..... *M. schulzeri* var. *tritici*

3a. Conidia mostly less than 10 µm long 4
3b. Conidia up to 14 µm long ..... *M. banksiae*

4a. Conidia rounded at the base ..... *M. flexuosum*
4b. Conidia slightly acuminate at the base ..... *M. phragmitis*
4c. Conidia tapering to a subtruncate base ..... *M. schulzeri* var. *schulzeri*

Acknowledgments

The authors are grateful for pre-submission comments and suggestions provided by Drs. R.F. Castañeda-Ruiz and De-Wei Li. This project was supported by the National Science Foundation of China (No. 31060005) and the Doctors' Funding of Guizhou University (no. 2010014). We are indebted to Prof. Tian-Yu Zhang (Shandong Agricultural University, China) for supplying the culture of this new species.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.9

Volume 124, pp. 9–20 *April–June 2013*

The occurrence of *Rutstroemia coracina* on *Quercus ilex* leaves in Spain

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Abstract— The authors report new findings of an apparently rare fungus, *Rutstroemia coracina* (*Rutstroemiaceae*). The species grew on old fallen leaves of *Quercus ilex* subsp. *ballota* at different localities in Guadalajara and Madrid provinces (center of Spain) where it was first collected in 1983 and subsequent years. The apothecia are described and illustrated in detail. Microscopic studies, including microtome sections, show a clear gel layer in the ectal excipulum, which allows assignment in the genus *Rutstroemia*. Since the type material deposited in PC is so scanty and deteriorated that any future revision will be impossible, an epitype is selected here.

Key words— *Ascomycota*, *Helotiales*, foliicolous fungi, taxonomy

Introduction

Palmer (1994) was the first to report the presence of *Rutstroemia coracina* from the European continent. This species was initially recorded in Algeria and was documented only by a coloured plate as "*Peziza coracina* DR. et Lév." in Durieu (1848: pl. 28 fig. 4) reproduced here as Fig. 1, which depicts apothecia (on a *Quercus ilex* leaf) with egg-shaped spores, paraphyses, and asci. Although no text accompanied the 1848 protologue, the figure caption published in Durieu (1869: 10) identified the substrate as the undersurface of a dried *Q. ilex* leaf and detailed the individual elements included in the figure. Later Saccardo (1889: 237) provided a brief description as *Helotium coracinum,* apparently based on the original figure since no microscopic measurements are given and enquiries by J.T. Palmer with herb. PAD (Università degli Studi di Padova) ascertained that there is no exsiccatum under this epithet in Herb. P.A. Saccardo.

The first authentic description seems to be by Dennis (1964: 39–40, fig. 12), who studied the type collection preserved in PC. Reporting ascospores "11–13 × 5–5.5 µm" and an "[e]xcipulum composed of parallel hyphae with glassy walls," he transferred the species to *Rutstroemia*.

Spooner (1981: 283, 285, fig. 22) redescribed the species from a few apothecia on a *Quercus ilex* leaf collected by M.C. Clark in 1977 near Somerset, southwest England, and recombined it in *Lanzia,* as he failed to find the "glassy walls" found by Dennis. He reported similar spore measurements to those mentioned by Dennis for the type.

In 1992, Palmer visited the Allerford plantation between Bossington and Porlock, Somerset, where Mr. Clark had collected his leaf, and collected large samples of dead *Q. ilex* leaves from different parts of the wood, which subsequently produced apothecia in natural culture. His measurements for the living spores (in tap water) were 12.4–16.8 (18.4) × 4.8–7.6 (8) µm.

Palmer (1994) also restudied the type remnants in PC and found a minute fragment (approximately haft an apothecium) among the stipes of immature apothecia on one of the two spiny leaves of the holotype; although it was not possible to make a detailed study, he managed to draw ovoid spores without any sign of guttulation (measuring 10.6–13.9 × 4.5–6.5 (7.3) µm), one ascus (measuring 106 × 12 µm), and scarce hyphae suspected to be from the gelatinous layer in the ectal excipulum protruding as obtuse "hairs" (3.3–6.0 µm wide) – reproduced here as Fig. 2. In addition, Palmer (1994: 226–228, figs 1–5) made good collections in northern Greece in March 1988, mainly on old, fallen leaves of *Quercus coccifera* and *Q. ilex* on Mount Athos and Mount Olympus but also on a leaf of *Quercus pubescens* (= *Q. humilis*) on Mt Athos.

This species was collected and identified for the first time in Spain by R.G. in 1983 on old, fallen leaves of *Quercus ilex* subsp. *ballota* at Tamajón (Guadalajara province). The identification was later confirmed by Palmer, who had the opportunity to record the species in subsequent years, including collections by Dr. A. Raitviir (Palmer & Galán 1999, Galán 2001). The area is open with scattered trees (intermixed with *Juniperus thurifera*), and leaf litter is sparse. More recently, during a field trip through Guadalajara as part of the XIII Congress of European Mycologists in Alcalá de Henares (September, 1999), several attendees had the opportunity to collect the species in abundance, including Drs. B. Spooner, M.M. Nauta and H.O. Baral, who kindly provided us with herbarium samples. More recent findings of the species by two of us (F.P.G. & A.G.) on old fallen leaves of *Quercus ilex* subsp. *ballota* at Colmenarejo (Madrid province) allow us not only to widen the distribution of *Rutstroemia coracina* in Spain but also to complete its description. In addition, Palmer (pers. comm.) also recorded and identified this species in 1990 on Minorca (Balearic Islands, Spain) growing on *Quercus ilex* leaves.

Fig. 1. Illustration of the type of *Peziza coracina* Durieu & Léveille, Expl. Sci. Algérie, Bot. 1: t. 28, f. 4 (1848).

From the beginning, Palmer (1994) found it noteworthy that the greyish green tinges of apothecia in both freshly collected material or that developed in damp chambers were overlooked in Spanish material, which more closely resembled the reddish brown color in the original Algerian plate. That might suggest the existence of two different taxa, but otherwise all specimens appeared to be microscopically identical.

Since the type material preserved in PC allows no further revision, we here select as epitype a very rich Spanish collection (about 30 fallen leaves of *Quercus ilex* subsp. *ballota,* bearing ca. 60–70 apothecia) whose habit, colour, and microscopic features (mainly ascospore shape and size) match the holotype very well.

There has been continued controversy since Dennis (1964) reported an "excipulum composed of parallel hyphae with glassy walls." This was why the taxon was first combined in *Rutstroemia* and why Spooner (1981) transferred the species to *Lanzia,* remarking, "I have not been able to find glassy walls." Spooner later had opportunity to re examine one of the Greek collections (J.T.P. 4418) identified as *R. coracina* and agreed entirely with Palmer's identification. He noted the presence of gelatinized tissue, which made him review the British material where he found "a gelatinous matrix to the ectal hyphae, something which I overlooked when referring the species to *Lanzia*" (B.M. Spooner, in letter). The microtome sections (Figs 6, 7) of English, Greek, and Spanish material show a clear gel layer in the ectal excipulum, although the thickness is not as prominent as in other *Rutstroemia* spp. and also is somewhat variable in width from collection to collection. Nonetheless, we feel that the species was correctly placed in *Rutstroemia* by Dennis (1964).

Rutstroemia coracina appears to be known only from Algeria (where the actual type locality is unknown), Britain (Spooner 1981), Greece (Palmer 1994), France (Palmer, pers. comm.), Turkey [K(M) 49709 Spooner, pers. comm.], and Spain.

Materials & methods

Measurements of living cells (marked *) were made in tap water or IKI (1% Lugol's solution), with dead cells (marked †) in tap water, Melzer's reagent (MLZ), KOH 2%, and aqueous cresyl blue (CRB) 0.5%. Line drawings were made using a Nikon Labophot-2 either by hand without the aid of a camera lucida (C.E.H.) or with a drawing tube incorporated into the light microscope (R.G.) with magnifications up to 1250×. Photomicrographs were made with using a Nikon Labophot-2 equipped with phase contrast and camera. Macroscopic pictures of fructifications in fresh state showing the general habit were taken in situ (by F.P.G.) or at the lab (by C.E.H.). Apothecial colours are cited according to Locquin (1985). Specimens have been deposited in AH and J.T.P. (personal herbarium of the late Mr. J. Terence Palmer, currently deposited in CUP) with additional specimens in H.O.B. (personal herbarium of Hans O. Baral, Tübingen, Germany) and K.

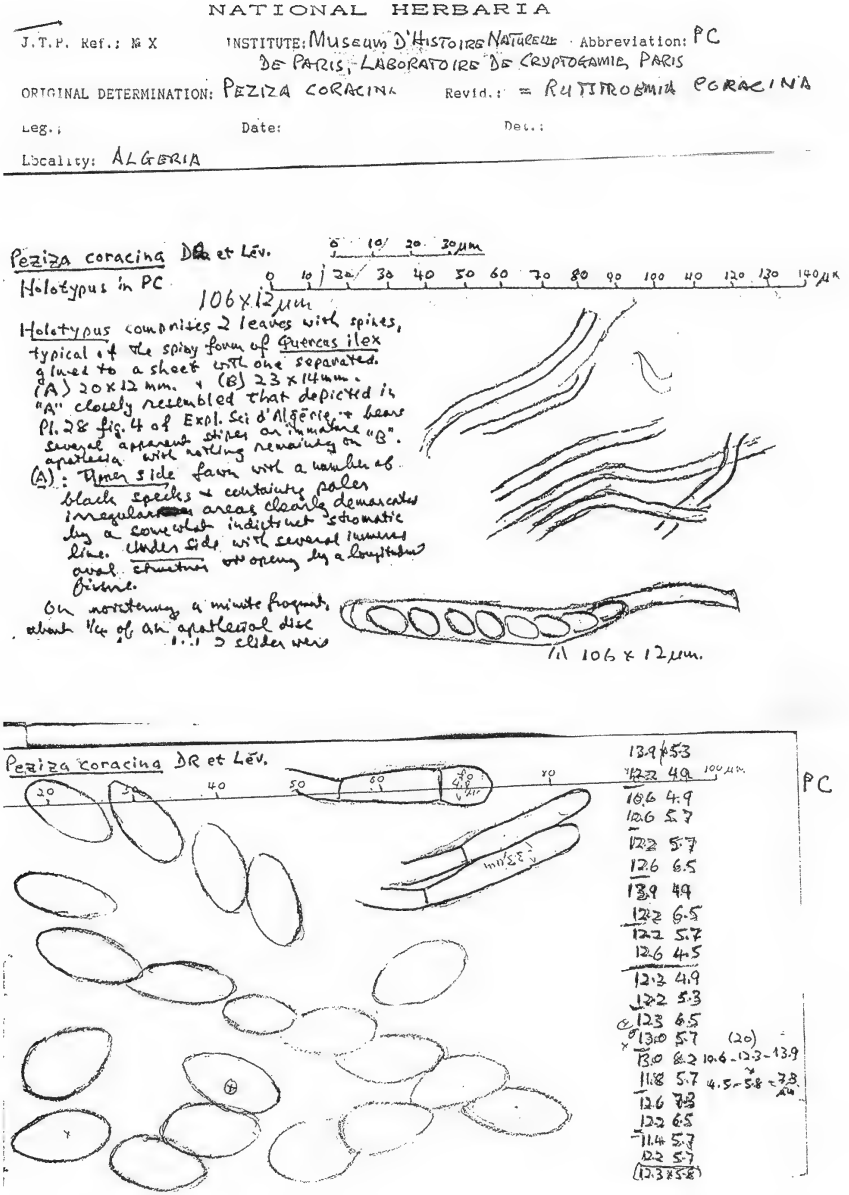

NATIONAL HERBARIA
J.T.P. Ref.: M X
INSTITUTE: Museum D'Histoire Naturelle · Abbreviation: PC
De Paris, Laboratoire De Cryptogamie, Paris
ORIGINAL DETERMINATION: Peziza coracina
Revid.: = Rutstroemia coracina
Leg.:
Date:
Det.:
Locality: Algeria
Peziza coracina DR et Lév.
Holotypus in PC
106 x 12 µm
Holotypus consists 2 leaves with spines, typical of the spiny form of Quercus ilex glued to a sheet with one separated. (A) 20 x 12 mm. & (B) 23 x 14 mm. "A" closely resembled that depicted in Pl. 28 fig. 4 of Expl. Sci d'Algérie. It bears several apparent stipes on immature "B". apothecia with netting remaining on (A): Upper side fawn with a number of black specks & containing paler irregular areas clearly demarcated by a somewhat indistinct stomatic line. Under side with several immersed oval structures eventually opening by a longitudinal fissure.
On sectioning a minute fragment about 1/4 of an apothecial disc
1/1 106 x 12 µm.
Peziza coracina DR et Lév.
PC

FIG. 2. *Rutstroemia coracina* (holotype, PC): Annotations and drawings by J.T. Palmer showing excipular hyphae and an ascus (up), ascospores, and "hyphoid" hairs (down).

Fig. 3. *Rutstroemia coracina* (AH 7641, epitype) growing in natural habitat.
Photo F.P.G. in situ (bar = 10 mm).

Fig. 4. Apothecia of *Rutstroemia coracina* (C.E.H. 08468) under high magnification.
Photo C.E.H. ex situ (bar = 1 mm).

Taxonomy

Rutstroemia coracina (Dur. & Lév.) Dennis, Persoonia 3: 39 (1964) FIGS. 1–7

≡ *Peziza coracina* Dur. & Lév., Expl. Sci. Algérie, Bot. 1: t. 28, f. 4 (1848)

≡ *Helotium coracinum* (Dur. & Lév.) Sacc., Syll. Fung. 8: 237 (1889)

≡ *Calycina coracina* (Dur. & Lév.) Kuntze, Revis. Gen. Pl. 3(3): 448 (1898)

≡ *Ciboria coracina* (Dur. & Lév.) Boud., Hist. Class. Discom. Eur.: 106 (1907)

≡ *Lanzia coracina* (Dur. & Lév.) Spooner, Trans. Br. Mycol. Soc. 76: 283 (1981)

TYPE: Algeria (**Holotype**, PC). Spain, Madrid, Colmenarejo, UTM 30TVK1489, 885 m a.s.l., on petioles, lower (more exceptionally upper) faces and veins of fallen leaves of *Quercus ilex* subsp. *ballota*, 27.XI.2011, Prieto & González, det. R.G. , rev. C.E.H. (**Epitype designated here**, AH 7641).

APOTHECIA superficial, scattered, cupulate, stipitate, castaneus to reddish-brown, merging from darkened areas of the substrate. DISC slightly concave becoming plane, flesh colour (i.e., russus B4f) to yellowish-olivaceous (i.e., venetus S2d), tending to darken with age, 0.5–2.0 mm in diameter. RECEPTACLE cupulate, covered with a sparse and irregular net of brown fibrils, vertically arranged with adpressed ends, sometimes protruding and giving a fringed appearance, colour similar to or concolorous with disc and stipe, sometimes chestnut (with varying reddish brown shades). MARGIN ochre, chestnut to reddish-brown, crenulate. STIPE equal in length to or longer than disc diameter, reddish-brown often almost black at the base, 0.3–3.0(–6.0) mm long. ECTAL EXCIPULUM composed of three layers: outer zone, 12–38 µm thick, formed of some sparse rows of loosely branched, flexuous, brown hyphae (3.7–5.5 µm in diam. †) arranged parallel to the surface, with thin walls encrusted with pigment giving a typically banded aspect and protruding as agglutinated "hairs" on flank and stipe or in groups as "clavate hairs" at the margin to form teeth; the medial layer of textura oblita, 15–60 µm thick, formed of flexuose, subhyaline to light pale ochre hyphae, arranged parallel or at a low angle to the surface, with very narrow lumina and strongly gelatinized walls; the inner zone of imprecise thickness, formed of prismatic to doliform cells (textura prismatica) and not well delimited from the medullary excipulum, hyphae 5–10 µm (†) in diameter, with pale chestnut content and thin walls. MEDULLARY EXCIPULUM well developed, of textura intricata and subhyaline cells with thin, smooth walls (3–5 µm in diam. †). SUBHYMENIUM compact, poorly developed and indistinguishable from the medullary area, apart from the brownish colour and highly intricate texture. ASCI 8-spored, cylindric-clavate, tapering at base to form a slender stalk, emerging from croziers, a round, truncate to fissured apex, with a long pore outlined J+ (deep blue) in both IKI and Melzer's reagent, 125–152 ×11–13 µm (*), 110–130(–140) ×9–11 µm (†). SPORES biseriate to uniseriate or irregularly biseriate (in dead state), hyaline, ellipsoid to elliptic-

fusoid, slightly inequilateral, regularly unicellular, rarely one to three-septate when over-mature, exceptionally producing subglobose microconidia (2–2.5 µm * diam.) at one or both apices, with high number of globose lipid bodies (1–2 µm) occupying most of the cytoplasm in the living spores (multiguttulate), forming two large distinct apical drops in dead state by coalescence, measuring 14–17(–18) × 6–7.5 µm (*), 12–15(–16.8) × (5–)5.5–6.8(–7.2) µm (†). PARAPHYSES straight, filiform to subclavate ca. 2–2.5 µm diam. (*), enlarged to 4–5.5 µm (*) at the apex, sparsely septate, simple or branched below, equaling or scarcely exceeding the asci in length, bearing brown to reddish-brown vacuolar contents ("refractive vacuolar bodies" sensu Baral 1985) in the living state.

ADDITIONAL SPECIMENS EXAMINED: **SPAIN**, **GUADALAJARA**, **Tamajón**, surroundings of Ermita del Humilladero, on a fallen leaf of *Quercus ilex* subsp. *ballota* in an open mixed wood (with *Juniperus thurifera*) with scattered trees and leaf litter sparse on the ground, 18.XI.1983, Galán, Moreno & Checa, det. R.G., rev. Palmer (AH 6235; duplicate, J.T.P. as permanent slides); on stromatized petioles and veins of fallen old *Quercus ilex* subsp. *ballota* leaves, 3.XII.1984, Galán, Moreno & Palmer, det. J.T.P. (J.T.P. 4125); 3.XII.1991, Galán & Raitviir, det. R.G. & J.T.P. (AH 6720; duplicate, J.T.P. 4695). **Cifuentes**, between Oter and Carrascosa de Tajo, on fallen leaves of *Quercus ilex* subsp. *ballota*, 25.IX.1999, Nauta & Spooner, det. J.T.P. (J.T.P. 4978; duplicates, AH 7334, K). **Mirabueno**, surroundings, 1000 m a.s.l., on side and veins of fallen leaves of *Quercus ilex* subsp. *ballota* lying on ground, 24.IX.1999, Baral & Marson, det. Baral, rev. J.T.P. (J.T.P. 4977; duplicates, AH 7335, H.O.B. 6477E). **MADRID**, **Colmenarejo**, UTM 30TVK1489, 885 m a.s.l., on petioles, lower (more exceptionally upper) faces and veins of fallen leaves of *Quercus ilex* subsp. *ballota*, 18.XI.2011, det. R.G. (AH 7640); 11.XII.2011, det. F.P., rev. C.E.H. (C.E.H. 08468). **ENGLAND, SOMERSET,** between **Allerford** and **Bossington**, on old, fallen leaves of *Quercus ilex* sampled, (apothecia developed in damp chamber from 26.IV–30.V.1992), 20.III.1992, Palmer, det. J.T.P., rev. R.G. (J.T.P. 4713; duplicate, AH 6813); (J.T.P. 4714; duplicate, AH 6812). **GREECE, MACEDONIA**, **Athos Mountain**, surroundings of Monastery Iveron, on stromatized fallen leaves of *Quercus ilex* subsp. *ilex*, 25.III.1988, Palmer, det. J.T.P., rev. R.G. (J.T.P. 4415; duplicate, AH 6604).

COMMENTS—There are at least three additional *Rutstroemia* species usually or occasionally reported from decaying *Quercus* leaves: *R. sydowiana* (Rehm) W.L. White (widely distributed at the Mediterranean basin) with "allantoid" spores (nearly ovoid, with basally bent beaks); *R. petiolorum* (Roberge ex Desm.) W.L. White (typically reported on *Fagus* leaves in Europe but occasionally also appearing on *Quercus* leaves) with allantoid to reniform spores with small polar gel caps; and *R. latispora* K.S. Thind & Saini, later transferred to *Lambertella* (Spooner 1987), found on *Quercus incana* leaves in the north-west Himalayas, having asymmetrical and much broader ascospores and longer asci than the two previous species (Thind & Saini 1968). In addition *Rutstroemia "kalevi"* Baral (ined.), which that could be misidentified as *R. coracina* (despite its *Mollisia*-like vacuoles in the paraphyses and narrower spores) grows on stromatized veins and petioles of *Acer* spp. in Central Europe (Baral & Marson 2005).

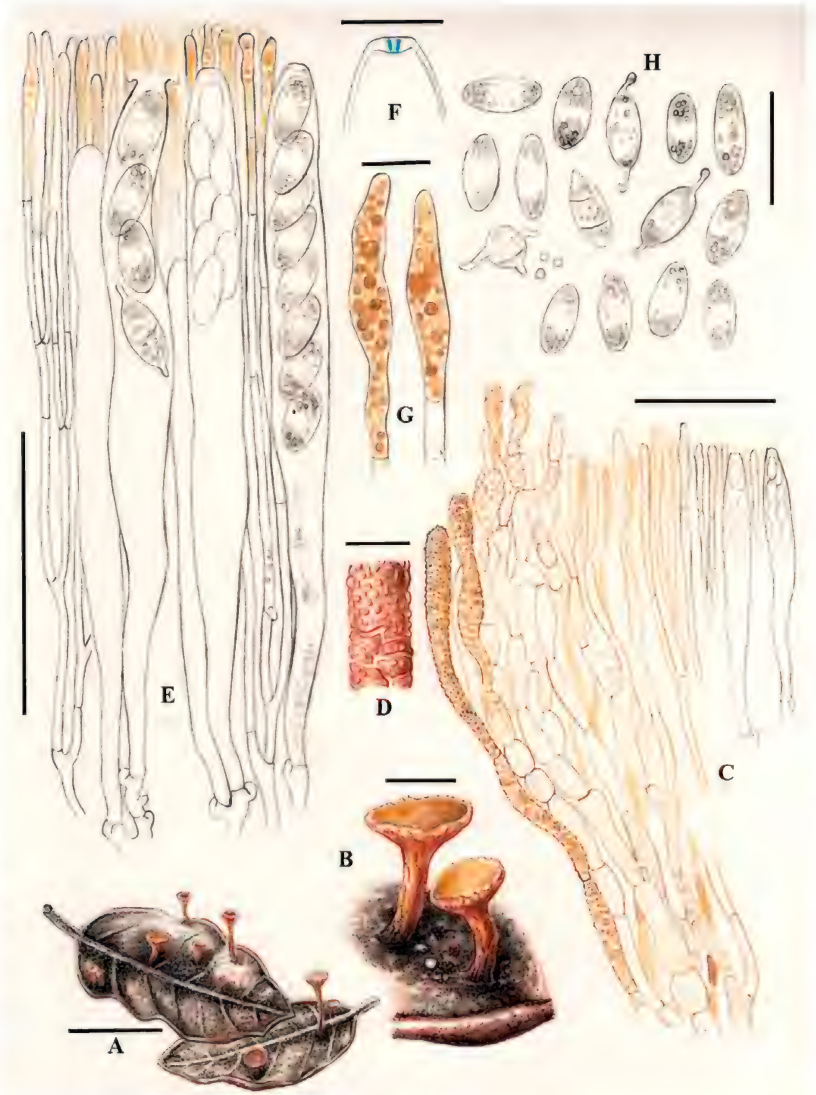
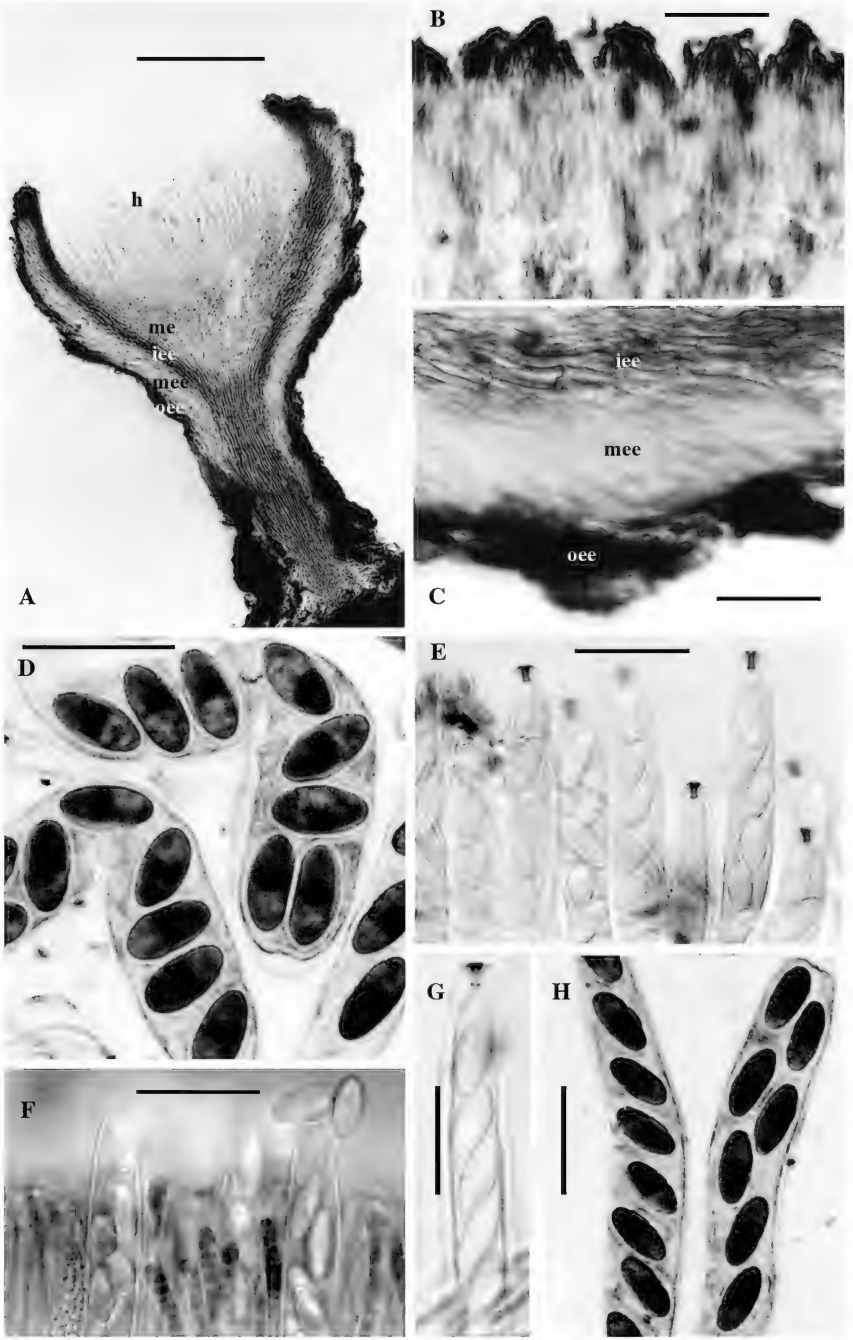


Fig. 5. *Rutstroemia coracina* (AH 7641, epitype): A. Apothecia in their natural habitat (bar = 10 mm); B. Enlarged view of fresh apothecia (bar = 1 mm); C. Cross section of an apothecium (marginal region) (bar = 50 µm); D. Encrusted wall of hyphae of the outer ectal excipulum (bar = 5 µm); E. Hymenium showing asci arising from croziers and paraphyses (bar = 50 µm); F. apex of a mature ascus with an euamyloid ring (bar = 10 µm); G. Paraphyses containing many refractive vacuolar bodies in apical cells (bar = 5 µm); H. Free 0-3-septate ascospores, exceptionally forming small conidia on germ tubes (bar = 20 µm). Mountants: tap water, except fig. F (IKI). Watercolour by C.E.H.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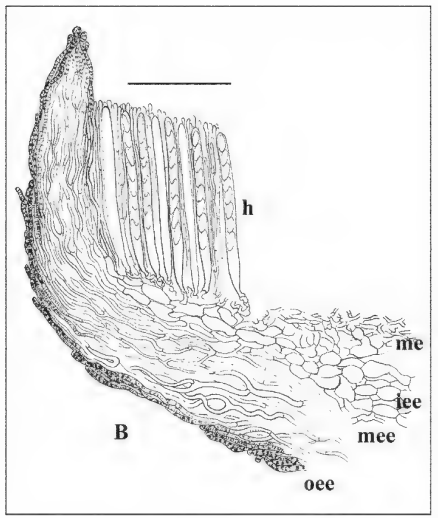

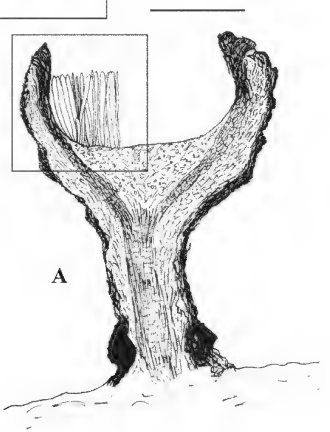

FIG. 6 (left). *Rutstroemia coracina*: A. Radial section of an apothecium (AH 6812; bar = 200 µm); B. Frontal view of the outer ectal excipulum forming marginal teeth (AH 6604; bar = 50 µm); C. Radial section of an apothecium at middle flanks showing a three layered ectal excipulum (AH 6812; bar = 50 µm); D, E, G, H. Asci with ascospores (AH 6235; bar = 20 µm for D, G, H; 25 µm for E); F. Mature asci, free spores and tips of paraphyses (AH 7641; bar = 25 µm). Mountants: 2% KOH (figs. A, B & C); CRB (figs. D & H); MLZ (figs. E & G); tap water (fig. F). Dead state except fig. F. Photographs by R.G. & C.E.H. (fig. F). Abbreviations: outer (= oee), medial(= mee) and inner (= iee) layers of the ectal excipulum; medullary excipulum (= me).

FIG. 7 (above). *Rutstroemia coracina* (AH 6812): A. Radial section of an apothecium (bar = 200 µm); B. Enlarged view of an apothecium (bar = 100 µm), at flank and margin, showing: the outer (= oee), medial(= mee) and inner (= iee) layers of the ectal excipulum; medullary excipulum (= me); hymenium (= h). Dead state (KOH 2%). Drawings by R.G. & C.E.H.

Acknowledgements

We would like to thank Dr. Richard P. Korf for suggestions and improvement in English grammar. Also to A. Pueblas of the "Gabinete de Dibujo y Fotografía Científica" at the Alcalá University for their invaluable help in the digital preparation of the photographs. We are grateful to Dr. Scott LaGreca (CUP), Dr. J. Rejos (AH), Dr. B.M. Spooner & Dr. B. Aguirre-Hudson (K), Mme J. Perreau (PC) for providing institutional specimens data, and Dr. M.N. Nauta and H.O. Baral for information on *Rutstroemia coracina* in their personal herbaria. One author (R.G.) extends his gratitude to the late Mr. J.T. Palmer for invaluable help with sclerotiniaceous fungi and a lifetime friendship. Finally, we wish to express our gratitude to Dr. S. Huhtinen and Dr. W.-Y. Zhuang for reviewing the manuscript and for their useful comments.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.21

Volume 124, pp. 21–29 *April–June 2013*

Two species of *Cladosporium* associated with wood discoloration in Korea

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Abstract — During our previous study on the diversity of molds inhabiting wood, many *Cladosporium* isolates were collected and phylogenetically identified. Among them were isolates of two species previously unrecorded from Korea. Here, we confirm the isolates as *Cladosporium perangustum* and *C. ramotenellum* and provide descriptions and illustrations of cultural and microscopic characters on standard media. We draw attention to a broadening of the range of several characters for *C. ramotenellum* and to possible alternative phylogenetic relationships of *C. perangustum* when comparing our multi-gene analysis with previous analyses.

Key words — ascomycetes, phylogeny, taxonomy

Introduction

Cladosporium Link is one of the most widely known (if under-studied) molds. It is a cosmopolitan genus with more than 772 names (Dugan et al. 2004) and recently Bensch et al. (2012) recognized a total of 169 *Cladosporium* species. It has been isolated from air, soil, and many other substrates (Schubert et al. 2007, Bensch et al. 2010, 2012). Members are variously known to be saprobic, endophytic, or plant pathogenic (Schubert et al. 2007, Bensch et al. 2010, 2012). In Korea, about 20 *Cladosporium* species have been reported (Min 1985, Shin & Braun 1995, Shin & Im 1999, Kwon et al. 2000, 2001, Paul & Yu 2008, Korean Society of Plant Pathology 2009, Bensch et al. 2010). Recently Lee et al. (2012) phylogenetically examined *Cladosporium* representatives inhabiting wood products in Korea and recognized nine *Cladosporium* species. Among them were *C. perangustum* and *C. ramotenellum*, not previously recorded in Korea. Here, we confirm their identities by morphology and phylogenetic re-analysis and provide detailed descriptions and figures for these two *Cladosporium* species.

Materials & methods

Morphological examination

Four *Cladosporium* isolates, KUC1462, KUC1767, KUC3027, and KUC5085 studied by Lee et al. (2012) were used in this study. They were inoculated onto SNA (Synthetic nutrient-poor agar: KH_2PO_4 1 g, KNO_3 1 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; KCl 0.5 g; glucose 0.2 g; sucrose 0.2 g; agar 20 g; distilled water 1000 ml) and cultivated at 25°C for 7 days. Preparations were mounted in Shear's solution (CH_3COOK 3 g; distilled water 150 ml; glycerin 60 ml; 95% ethanol 90 ml) according to Crous et al. (2009). Microscopic observations were made using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) and photos were taken using the same microscope and an Axio Imager A1 microscope (Zeiss, Jena, Germany). For determination of cultural characteristics, colonies were cultivated on PDA (Potato dextrose agar, Difco), MEA (Malt extract agar: malt extract 30 g; agar 15 g; distilled water 1000 ml), and OA (Oatmeal Agar, Difco) at 25°C for 14 days in the dark. The isolates were deposited at the National Institute of Biological Resources, Incheon, South Korea (KB) with the acronym KUC, which refers to the Korea University Culture Collections, Korea University, Seoul, South Korea.

Phylogenetic analysis

DNA sequences of four *Cladosporium* isolates (KUC1462, KUC1767, KUC3027, KUC5085) from the internal transcribed spacer (ITS), actin (ACT), and translation elongation factor-1α (TEF) regions were obtained from Lee et al. (2012). ITS, ACT, TEF sequences were aligned separately with the reference sequences from Bensch et al. (2010) using MAFFT 6.885 (Katoh & Toh 2008). The L-INS-I alignment option was used for all datasets. Each dataset was manually edited with MacClade 4.08 (Maddison & Maddison 2005). Gaps were treated as missing. The best-fit model was applied for each dataset using AIC in MrModeltest 2.3 (Nylander 2004) and the three datasets were combined. Bayesian analysis was performed with the combined dataset using MrBayes 3.2.1 (Ronquist et al. 2012). Two independent runs (four chains each) of 705,000 generations were performed and every 100th tree was sampled. The first 25% of sampled trees were discarded and the remaining 75% were used for construction of a 50% major-rule consensus tree. The graphic representation of the likelihood scores of the sampled trees was checked. The standard deviation of split frequencies is below 0.01 and the potential scale reduction factor (PSRF) was close to 1.0. The tree was viewed via FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Taxonomy

Cladosporium perangustum Bensch, Crous & U. Braun, Stud. Mycol. 67: 65. 2010.
FIGS 1–2

Mycelium internal and superficial; 1.5–4 µm wide, septate, sometimes slightly constricted at septa, sometimes intercalary swellings and constrictions present, subhyaline or pale olivaceous-brown, smooth or verruculose. Conidiophores macronematous or micronematous arising terminally and laterally from hyphae, straight or slightly curved, filiform to narrowly cylindrical-oblong, unbranched, occasionally branched, conidiophores 14–142 × 2–3.5 µm, pale olivaceous-

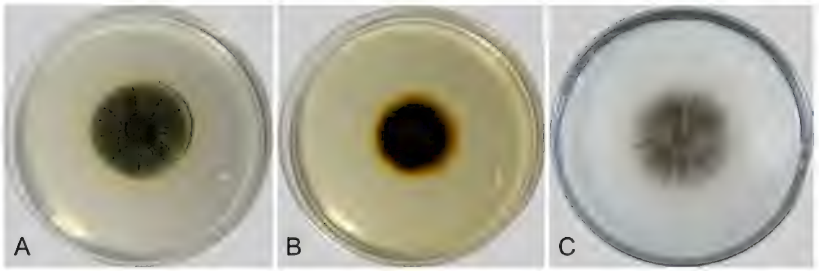

Fig. 1. *Cladosporium perangustum* (KUC1767) grown in 9 cm diam Petri dishes for 14 d at 25°C. A: PDA. B: MEA. C: OA.

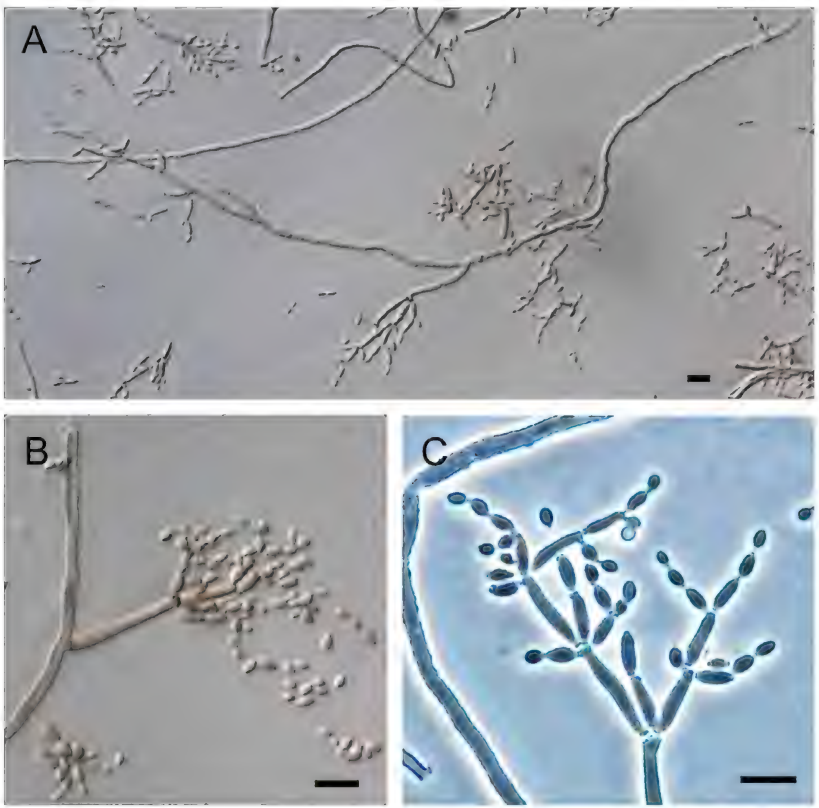

Fig. 2. *Cladosporium perangustum*. A, B. Macro- and micronematous conidiophores (KUC1462). C. Ramoconidia and conidia (KUC1767). Scale bar = 10 μm.

brown, asperulate-verruculose towards the base of conidiophores, at the apex smooth, sometimes slightly attenuated towards the apex. Conidiogenous cells integrated, mainly terminal, sometimes also intercalary, narrowly cylindrical-

oblong, in intercalary cells loci situated on small peg-like lateral prolongations or just below the septum, 13–36 µm long, with 1–4 apically crowded loci. Ramoconidia cylindrical-oblong, up to 44 µm long, 2.5–3.5 µm wide, rarely septate, base truncate, secondary ramoconidia narrowly ellipsoid to cylindrical-oblong, 8–21 × 2–3 µm (av. ± SD: 13.7 ± 4.1 × 2.5 ± 0.2), with 2–4 distal hila, pale olivaceous-brown. Conidia numerous, catenate, in branched chains, branching in all directions, 1–4 conidia in the terminal unbranched part of the chain, small terminal conidia globose, subglobose or ovoid to obovoid, 2–3.5 × 1.5–2 µm (av. ± SD: 2.9 ± 0.4 × 1.8 ± 0.2), intercalary conidia ovoid, limoniform to ellipsoid, somewhat fusiform, 4–8.5 × 2–3 µm (av. ± SD: 5.9 ± 1.4 × 2.2 ± 0.2), attenuated towards apex and base, with 1-3 distal hila, pale olivaceous-brown.

Culture characteristics Colonies on PDA attaining 32–38 mm diam after 14 d at 25°C, greenish grey to dull green, reverse olive or dark green, fluffy, floccose or powdery, margins glabrous, whitish, olive grey or pale regular or somewhat undulate, aerial mycelium loosely floccose or felty, occasionally numerous small to large prominent exudates formed, sporulation abundant. Colonies on MEA reaching 20–29 mm diam after 14 d at 25°C, dark green or brown, reverse olive brown, velvety to floccose, margins white to light yellow, narrow to broad, regular to undulate, glabrous, aerial mycelium abundantly formed, sometimes covering most parts of colony surface, loosely to densely floccose or felty, growth habit plane to sometimes elevated, sporulation abundant. Colonies on OA 35–49 mm diam after 14 d at 25°C, grey to dark green, fluffy to felty-floccose, margins colorless or white, up to 2 mm diam, aerial mycelium abundant, covering large parts of the colony surface, dense, low to high, white, sporulating profusely.

Specimens examined: **KOREA**, **Gyeongsangbuk-do**, Bonghwa-gun, on the surface of *Pinus densiflora* log, 05 June 2001 (KB, KUC1462; GenBank JN033481, JN033508, JN033536); **Gyeongsangnam-do**, Pusan, on the surface of *Pinus radiata* log, August 2000 (KB, KUC1767; GenBank JN033468, JN033495, JN033523); **Incheon**, on the log surface of *Picea abies*, 16 June 2007 (KUC5085; GenBank JN033460, JN033487, JN033515).

Remarks — The colony morphology and microscopic features of the isolates agreed well with the description of *C. perangustum* (Bensch et al. 2010). The fungus has been reported from Africa, Asia, Australasia, Europe, and North America. According to Bensch et al. (2010), *Cladosporium exile* Bensch et al. and *C. scabrellum* Bensch et al. are similar to *C. perangustum* but *C. exile* has longer and wider conidiophores, wider ramoconidia and conidia, and shorter intercalary conidia. *Cladosporium scabrellum* differs in having mainly macronematous, wider conidiophores, and wider secondary ramoconidia. Compared to the other species studied by Lee et al. (2012), *C. perangustum* showed medium discoloration on *Pinus densiflora* and *Pinus radiata*.

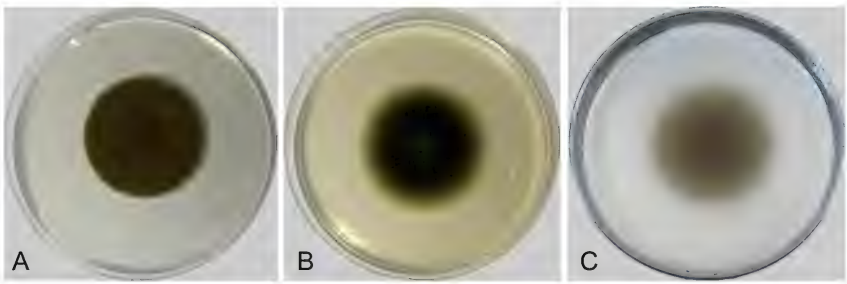

Fig. 3. *Cladosporium ramotenellum* (KUC3027) grown in 9 cm diam Petri dishes for 14 d at 25°C. A: PDA. B: MEA. C: OA.

Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun, Stud. Mycol. 58: 137. 2007. Figs 3–4

Mycelium internal and superficial, 1.5–4 µm wide, septate, without swellings and constrictions, hyaline or subhyaline, smooth. Conidiophores macronematous and micronematous, arising terminally or laterally from branches of plagiotropous hyphae, straight or slightly flexuous, oblong or cylindrical, unbranched, sometimes branches often only as short lateral prolongations, 15–117 × 2–3.5 µm, septate, pale olivaceous-brown or brown, smooth to verruculose. Conidiogenous cells integrated, terminal, sometimes also intercalary, cylindrical, 15–49 µm long, proliferation sympodial, with few conidiogenous loci, mostly 1–3, loci sometimes situated on small lateral prolongations. Ramoconidia cylindrical-oblong, up to 46.5 µm long, 2–3.5 µm wide, usually 0–1-septate, pale olivaceous, smooth or verruculose, with a broadly truncate base, secondary ramoconidia subcylindrical to cylindrical-oblong, 12.5–22 × 2–4 µm (av. ± SD: 18 ± 4 × 3.1 ± 0.5), pale olivaceous, apex broadly rounded or slightly attenuated towards apex and base. Conidia numerous, catenate, in branched chains, branching in all directions, small terminal conidia numerous, 1–3 conidia in the terminal unbranched part of

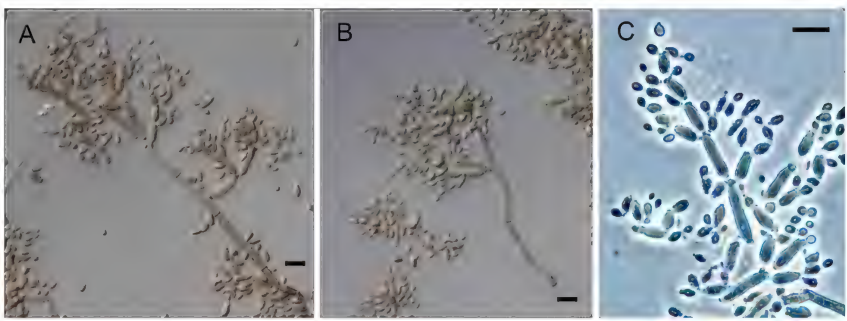

Fig. 4. *Cladosporium ramotenellum* (KUC3027). A, B. Macro- and micronematous conidiophore. C. Ramoconidia and conidia. Scale bars = 10 µm.

the chain, globose, subglobose or ovoid, obovoid, 3.5–7 × 2–3 μm (av. ± SD: 4.3 ± 0.7 × 2.5 ± 0.3), intercalary conidia ellipsoid to subcylindrical, or limoniform, 4–11 × 2.5–4(–4.5) μm (av. ± SD: 6.6 ± 1.6 × 3 ± 0.3).

Culture characteristics Colonies on PDA reaching 41–47 mm diam after 14 d at 25°C, olive due to abundant sporulation, margins entire, white, glabrous, aerial mycelium absent or sparse, growth flat with a somewhat folded and wrinkled colony center, no exudates, sporulation profuse. Colonies on MEA reaching 46–49 mm diam after 14 d at 25°C, olive, velvety, greyish green to dark green in reverse, margins entire, colorless, glabrous to feathery, aerial mycelium sparse, diffuse, growth flat with slightly elevated colony center, prominent exudates not formed, abundantly sporulating. Colonies on OA attaining 39–41 mm diam after 14 d at 25°C, olivaceous, margin entire, colorless or white, aerial mycelium sparse, growth flat, without exudates, sporulation profuse.

Specimen examined: **KOREA**, on the surface of Chromated Copper Arsenate (CCA)-treated wood product (*Pinus radiata*), 2003 (KUC3027; GenBank JN033464, JN033491, JN033519).

Remarks — The colony morphology and microscopic features of our isolate agreed well with the description of *C. ramotenellum* (Schubert et al. 2007), although it has smaller intercalary conidia and secondary ramoconidia. The fungus previously has been reported only from Slovenia. According to Schubert et al. (2007), *C. ramotenellum* resembles *C. cladosporioides* (Fresen.) G.A. de Vries and *C. tenellum* K. Schub. et al. but differs from *C. cladosporioides* by having narrower conidiophores and conidia. *Cladosporium tenellum* possesses conidiophores with numerous conidiogenous loci, and shorter and wider conidia. Also, *C. ramotenellum* grows faster in culture than *C. tenellum*. *Cladosporium ramotenellum* showed a discoloration rate similar to *C. perangustum* on *Pinus densiflora*, but a little bit more prominent than *C. perangustum* on *Pinus radiata* (Lee et al. 2012).

Phylogeny

The resulting alignment contained 36 taxa (including the outgroup taxon) and 481, 139 and 201 characters (including alignment gaps) were used in the ITS, ACT and TEF partitions, respectively. The model used for ITS was SYM+G model. For ACT and TEF, HKY+I+G model and GTR+G model were used, respectively. For the Bayesian analysis, 10,705 trees were obtained from which the consensus tree and posterior probabilities were calculated (Fig. 5). The species relationships in the tree were sometimes not as well resolved as in previous studies, or resolved differently (Bensch et al. 2010, Bensch et al. 2012). The phylogenetic analysis revealed that *Cladosporium perangustum* isolates, KUC1462, KUC1767 and KUC5085 were clustered within the *C. perangustum* species clade with a high posterior probability value (1.0

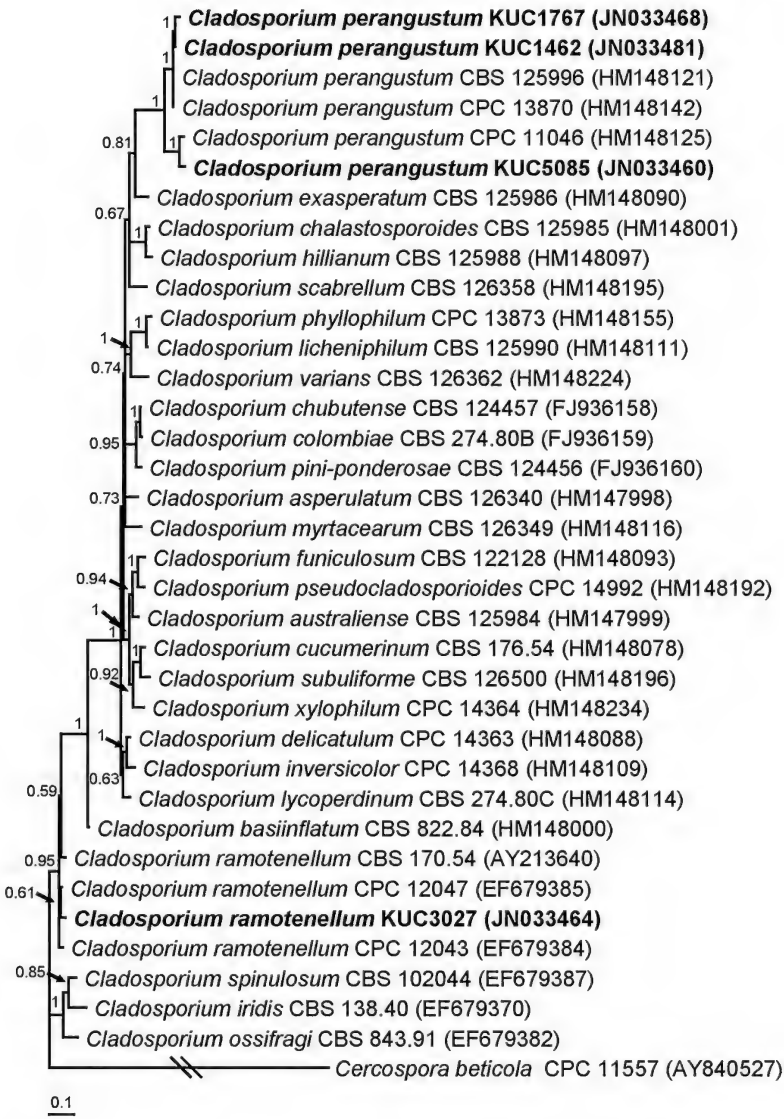

FIG. 5. 50% majority-rule consensus tree of 10,575 trees resulting from a bayesian analysis of 36 sequences in a combined ITS, ACT, and TEF alignment. Bayesian posterior probabilities ≥50% are shown. The tree was rooted to sequences of *Cercospora beticola* strain CPC 11557. GenBank Accession numbers of ITS sequences are shown in parentheses.

p.p.). In our analysis, *C. perangustum* was sister to *C. exasperatum* Bensch et al., a result differing substantially from the analysis of Bensch et al. (2010). In our analysis, *Cladosporium ramotenellum* KUC3027 was monophyletic with *C. ramotenellum* CPC 12047, but not with *C. ramotenellum* CPC 12043 and CBS 170.54. Also the monophyly of *C. ramotenellum* KUC3027 and CPC 12047 was weakly supported (61% posterior probability value). Nevertheless, the cultural and microscopic observations of our *C. ramotenellum* KUC3027 were consistent with the descriptions of *C. ramotenellum* (Schubert et al. 2007; Bensch et al. 2010).

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10011390) and was funded by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea. We are grateful to Dr. Pedro Crous and Dr. F.M. Dugan for their valuable suggestions on the manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.31

Volume 124, pp. 31–37 *April–June 2013*

Phialophora avicenniae sp. nov., a new endophytic fungus in *Avicennia marina* in China

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Abstract — Three isolates of an endophytic fungus were obtained from *Avicennia marina*. Morphological and molecular evidence indicates that they are identical and represent a new species of *Phialophora*, described here as *P. avicenniae*. The new species is characterized by brown colonies, branched conidiophores, and catenulate conidia.

Key words — *Herpotrichiellaceae*, ITS sequence, taxonomy

Introduction

The morphologically vaguely delimited hyphomycete genus *Phialophora* Medlar is characterized by dark and slow-growing colonies, pigmented hyphae, solitary or aggregated conidiophores, and flask-shaped phialides usually with a flaring collarette. The type of *Phialophora* is *P. verrucosa* Medlar, diagnosed by its slow-growing dark olivaceous colonies and usually pigmented phialides bearing an even darker collarette (Medlar 1915). *Phialophora* has been regarded as highly polyphyletic, comprising anamorphs of discomycetes, pyrenomycetes, and loculoascomycetes (Gams 2000). Gams & Holubová-Jechová (1976) distinguished sect. *Catenulatae* with catenate conidia having a truncate base. Some members of this section and other phialophora-like species with catenulate conidia, such as *Brachyalara straminea* Réblová & W. Gams, *Exochalara longissima* (Grove) W. Gams & Hol.-Jech., and *Infundichalara microchona* (W. Gams) Réblová & W. Gams, have been segregated from *Phialophora* by molecular phylogeny analyses and morphological characters (Bogale et al. 2010; Réblová et al. 2011). *Phialophora* s. str. is recognized as a member of the *Herpotrichiellaceae* (Untereiner et al. 1995).

We obtained three isolates of an endophytic fungus from twigs of *Avicennia marina* (Forssk.) Vierh. (*Acanthaceae*) in Zhanjiang, Guangdong Province, China, in 2007. The taxonomic status of these isolates was determined using morphological and molecular methods, showing them to represent a new *Phialophora* species. The isolates are stored in the laboratory of the Agricultural College, Guangdong Ocean University, and subcultures at CBS in Utrecht, the Netherlands.

Materials & methods

Isolates

Healthy twigs (0.4–0.8 cm diam.) of *Avicennia marina* were thoroughly washed in running tap water and then sterilized by washing in 75% ethanol for 2 min and 0.1% mercuric chloride (v/v) for 2 min. The twigs were rinsed 3 times in sterile distilled water and then cut into pieces (0.5 cm in length). The small pieces were evenly spaced in Petri dishes (9 cm diam) containing a medium (Arnold et al. 2000) consisting of (per litre distilled water) dextrose 20 g, peptone 10 g and agar 20 g, with addition of 60 mg streptomycin before pouring the plates. The Petri dishes were incubated at 27°C. Hyphal tips were transferred to potato dextrose agar (PDA) dishes.

Morphological examination

Three cultures of every isolate grown on PDA plates were investigated for colony characteristics. Thirty conidia were measured under an Olympus-BX51 microscope. Scanning electron microscopy (SEM) was used to observe phialide morphology. Specimens were flash-frozen (–196°C) in liquid nitrogen under vacuum for cryo-SEM, transferred to the preparation chamber, and then to the SEM chamber where ice particles were sublimated (–80°C). Samples were sputter-coated with E-1010 in the preparation chamber for 75 sec under 2.0 KV at –170°C. Specimens were viewed under 5 KV at –188°C with a Philips SL30 scanning electron microscope.

DNA extraction, PCR amplification, DNA sequencing, and phylogenetic analyses

After the isolates were grown and incubated on PDA plates at 27°C for 10 days, the mycelia were scraped off. Genomic DNA was extracted from the mycelia using SDS procedure (Lee & Taylor 1990) with a few modifications. The quality and quantity of DNA were visually assessed by staining with ethidium bromide in 1% agarose gel electrophoresis. The partial ITS sequences were amplified according to Geiser et al. (2005) using primers ITS1 and ITS4 (White et al. 1990) in TP600 (TaKaRa PCR Thermal Cycler Dice). Shanghai Invitrogen Biotechnology Co., Ltd. sequenced the DNA. The ITS sequences of the isolates were aligned using MEGA version 4.1 (Tamura et al. 2007) and manually optimized to ensure positional homology. Gaps were considered as missing data. Seventeen sequences of *Phialophora* and one sequence of an unidentified ascomycotan species were downloaded from GenBank, and *Capronia semiimmersa* (*Herpotrichiellaceae*) was chosen as outgroup. A phylogenetic tree was constructed using the neighbor-joining method of MEGA version 4.1 (bootstrapped 1000 replicates).

Taxonomy

Phialophora avicenniae Yue L. Liu & Z.D. Jiang, **sp. nov.** FIGS. 1–2

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Differs from *Phialophora clavispora* and *P. olivacea* by its branching conidiophores and endophytic habit and from *P. oxyspora* by its ovoid to globose conidia and endophytic habit.

TYPE: China, Guangdong Province, Zhanjiang, endophytic in twig of *Avicennia marina*, 18 April 2007, Liu Yuelian Q37 (**Holotype**, CBS 130286 [lyophilized culture]; GenBank, GQ500118).

ETYMOLOGY: named after the host genus, *Avicennia*.

COLONIES initially yeast-like, gradually becoming cottony, brown, reaching 2–3 cm diam. after 5 days at 27°C on PDA plates; reaching 9 cm diam. after 14

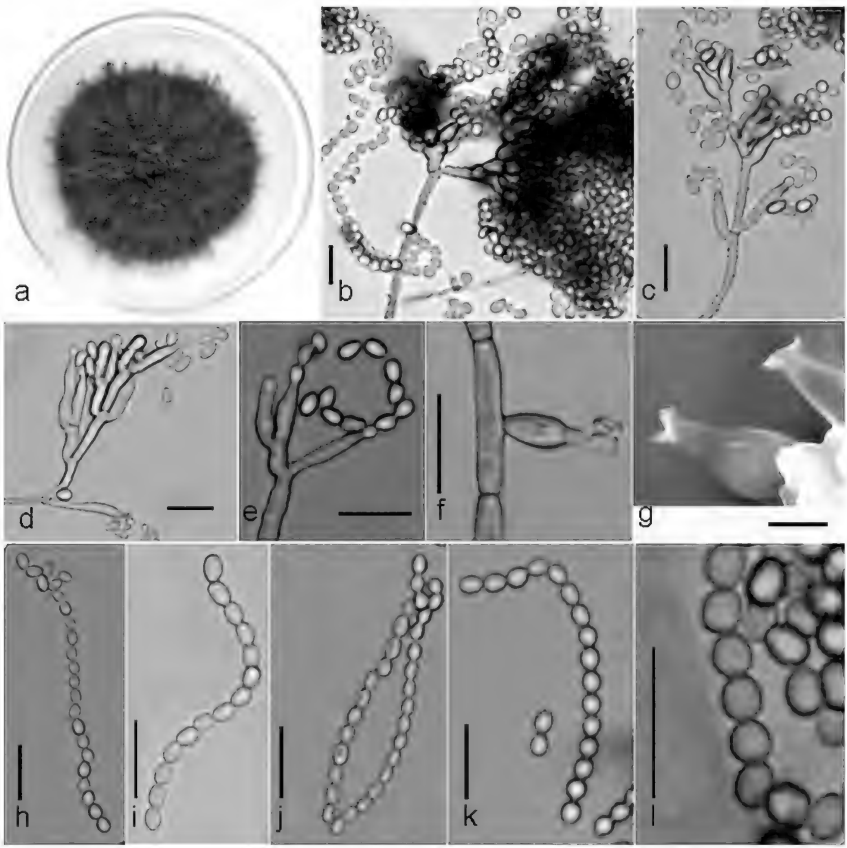

FIGURE 1. *Phialophora avicenniae* (CBS 1302876). a. Colony; b–f. Conidiophores; g. Phialides; h–l. Conidial chains. Scale bars: b–f, h–l = 10 μm; g = 2 μm.

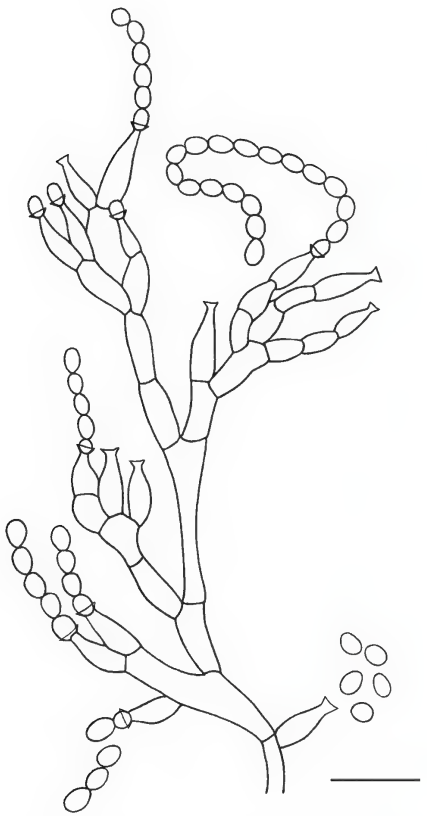

FIGURE 2. *Phialophora avicenniae.* Conidiophore and conidia. Bar = 10 μm.

days and changing from brown to black-brown. HYPHAE hyaline to subhyaline, smooth; occasionally brown, verrucose, 1.5–3.0 μm wide. CONIDIOPHORES hyaline or brown, branched, rarely simple, septate, smooth, and variable in size. PHIALIDES usually aggregated on conidiophores, flask-shaped or cylindrical, hyaline or brown, smooth and thick-walled, 6.5–11 × 1.8–3.5 μm. Collarette somewhat darker than the phialide body, funnel-shaped or flaring, 0.5–1.0 × 0.3–0.5 μm. CONIDIA catenulate, hyaline or brown, 1-celled, smooth, ovoid to globose, slightly apiculate at the base, 1.6–2.5 × 1.8–3.5 μm. CHLAMYDOSPORES absent.

TELEOMORPH: unknown

HABITAT: endophytic in twigs of *Avicennia marina*.

ADDITIONAL CULTURES EXAMINED: **CHINA. GUANGDONG PROVINCE**, Zhanjiang, endophytic in twigs of *Avicennia marina*, 10 August 2007, Liu Yuelian Q44 (CBS 130287; GenBank, HM055753); Liu Yuelian Q48 (CBS 130288; GenBank, HM055754).

NOTES: *Phialophora verrucosa*, the type of *Phialophora*, differs from the new species by olivaceous-black colonies and broadly ellipsoidal conidia adhering in slimy heads. The new species resembles *P. clavispora* W. Gams, *P. olivacea* W. Gams, and *P. oxyspora* W. Gams by its catenulate conidia. However, *P. clavispora* and *P. olivacea* have simple conidiophores, and *P. oxyspora* has fusiform conidia (Gams & Holubová-Jechová 1976). In addition, the new species is endophytic, while the other three species are so far only known as saprobes.

Phylogenetic analyses

ITS rDNA sequences obtained from the isolates of CBS 130286, CBS 130287, and CBS 130288 were submitted to GenBank and received the

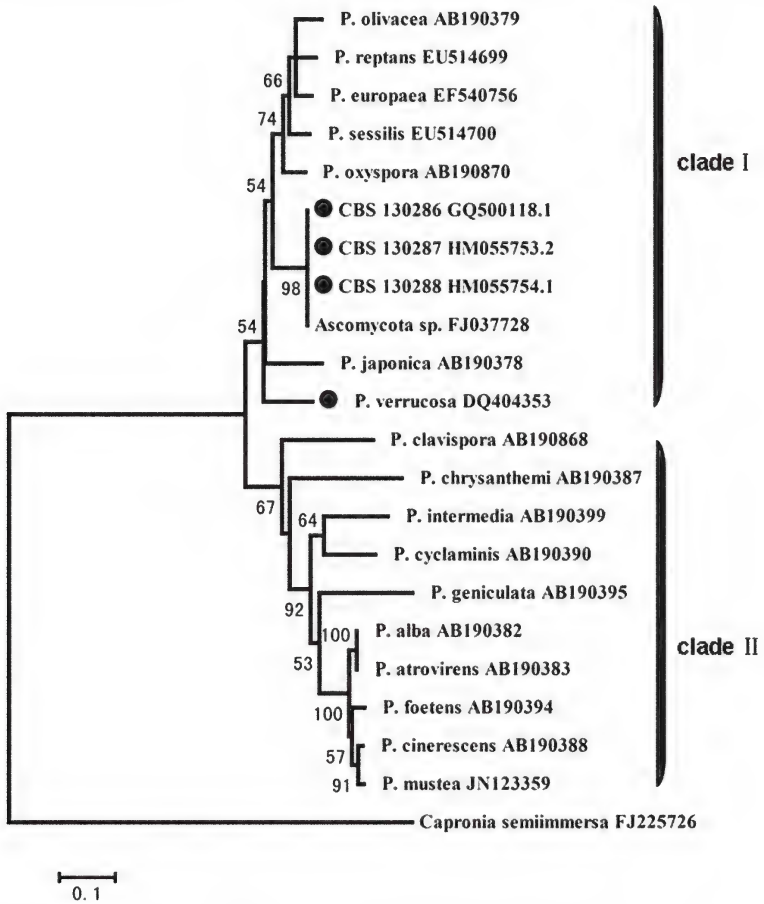

FIGURE 3. Phylogenetic tree based on neighbor-joining of ITS sequences, showing the relationships among *Phialophora avicenniae* and related species.

accession numbers GQ500118, HM055753, and HM055754. Blast (Basic Local Alignment Search Tool) analysis showed that the ITS sequences are 99% similar to an unidentified strain labeled "Ascomycota sp. GX6-1C" (GenBank FJ037728; isolated as an endophyte from an unknown mangrove host in China) and 79–87% similar to various GenBank isolates of *Phialophora* spp. A neighbor-joining analysis shows the relationships of the three strains with 17 other species of *Phialophora* (Fig. 3). The phylogram also shows that the unidentified strain forms a monophyletic clade (bootstrap 98%) with the three *P. avicenniae* strains, and is probably conspecific.

The *Phialophora* species shown here form two distinct clades (clade I and clade II) where *P. avicenniae* is part of clade I (bootstrap 54%), showing a sister relationship with *P. olivacea*, *P. reptans*, *P. sessilis*, *P. europaea*, and *P. oxyspora*.

Acknowledgements

We express our sincere thanks to Prof. W. Gams who revised the manuscript and deposited the strains in CBS. We also thank Prof. Yi-Jian Yao (Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences) for reviewing the manuscript. The authors are grateful to Dr. Martina Réblová (Department of Taxonomy, Institute of Botany, Academy of Sciences, Průhonice, Czech Republic) for providing valuable suggestions. This study was supported by the National Science Foundation of China (No. 30870015).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.39

Volume 124, pp. 39–45 *April–June 2013*

Ramichloridium strelitziae associated with sooty blotch and flyspeck on *Ravenala madagascariensis* in China

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Abstract — The first report of *Ramichloridium strelitziae* from China is documented. In a survey of host plants for SBFS fungi, we isolated the fungus from colonies on stems of *Ravenala madagascariensis* collected from Haikou, Hainan, China. It is distinguished from the other species in the genus by morphological characters and phylogenetic analysis based on ITS sequences.

Key words — microfungi, *Capnodiales*, taxonomy, phylogeny, traveller's palm

Introduction

Sooty blotch and flyspeck (SBFS) are epiphytes that colonize the waxy cuticle of a wide range of plants in humid regions worldwide. SBFS fungi can result in a black or sooty appearance leading to cosmetic damage that can cause significant economic losses (Batzer et al. 2005).

For approximately 70 years, SBFS was viewed as two distinct diseases, each caused by a single species of fungus. Recently, this concept was revised by combining morphological characterization with genetic analysis. Presently, it is evident that SBFS includes a spectrum of intergraded mycelial types and comprises more than 80 species (Batzer et al. 2008, Frank et al. 2010, Gleason et al. 2011, Li et al. 2012, Yang et al. 2010).

In this study, we identified one isolate that was described as the first record of *R. strelitziae* on stems of *Ravenala madagascariensis* Sonn. (*Strelitziaceae*; traveller's palm) from China based on morphological comparison and phylogenetic relationships.

= Joint first authors

Materials & methods

Isolates

Ravenala madagascariensis stems with flyspeck signs (Gleason et al. 2011) were found in Jinniuling Park in Haikou city, Hainan Province (20°00′26″N 110°20′31″E), China, in October 2011. Thalli on the stem were transferred directly from colonies to potato dextrose agar (PDA) slants in a sterile environment and cultured at 25°C for 1 month in darkness (Sun et al. 2003). Hyphal tips were then transferred to malt extract agar (MEA) plates. The isolates were allowed to grow onto an adjacent, sterile cover slip that had been partially inserted into the agar surface at a 60° angle in order to measure and observe fungal structures (Li et al. 2011). Microscopic examination was made after 14 d of incubation. Thirty measurements per relevant microscopic structure were gathered where possible. Colony descriptions (surface and reverse) were made after 2 weeks of growth on MEA plates at 25±1°C in the dark. The isolate acronym HKLRJ-4 was a temporary laboratory number. Representative dried culture and plant specimens were deposited in the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi Province, China.

DNA extraction, PCR, and phylogenetic analysis

Genomic DNA for polymerase chain reaction (PCR) was obtained according to the protocol of Li et al. (2011). The primer pair ITS1-F and ITS4 was used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The PCR reactions were carried out with Taq polymerase, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, and 2 µl of template DNA, and was made up to a total volume of 25 µl with sterile water. Reactions were performed on a Bio-Rad PCR System S1000TM Thermal Cycler. The amplification conditions were: initial denaturation at 94°C for 90 min followed by 35 cycles of denaturation at 94°C for 35 s, annealing at 52°C for 60 s, extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were sequenced by Sangon Biotech Co., Shanghai, China.

The ITS nucleotide sequence generated in this study was added to other sequences with high homology as the result of a BLAST search. *Cladosporium bruhnei* was used as the outgroup taxon. Sequences were imported into BioEdit 5.0.9.1 (Hall 1999). Preliminary alignments were performed using CLUSTAL-X (Thompson et al. 1997), then manually adjusted. Phylogenetic analysis of aligned DNA sequences was carried out with PAUP version 4.0b 10 for 32-bit Microsoft Windows (Swofford 2003). Heuristic searches were performed with 1,000 random sequence additions. Clade stability was assessed by 1,000 bootstrap replications. Other measures calculated for parsimony analysis included tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI).

The ITS sequence described in this study is deposited in GenBank as JX502176, and the alignment and representative tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13803).

Results

Phylogenetic analyses

The ITS alignment contained 29 taxa (including the outgroup) and 520 characters including alignment gaps. Of these characters, 266 were constant,

62 were variable and parsimony-uninformative, and 192 were parsimony-informative. One of the 10 equally most parsimonious trees saved from the maximum parsimony analysis is shown in Fig. 1. From the most parsimonious tree, two major clades were resolved. One clade, with 100% bootstrap support, contained fifteen species in *Ramichloridium* sensu stricto, *Uwebraunia*, *Dissoconium*, and *Pseudoveronaea* (all in *Dissoconiaceae*). The other major clade

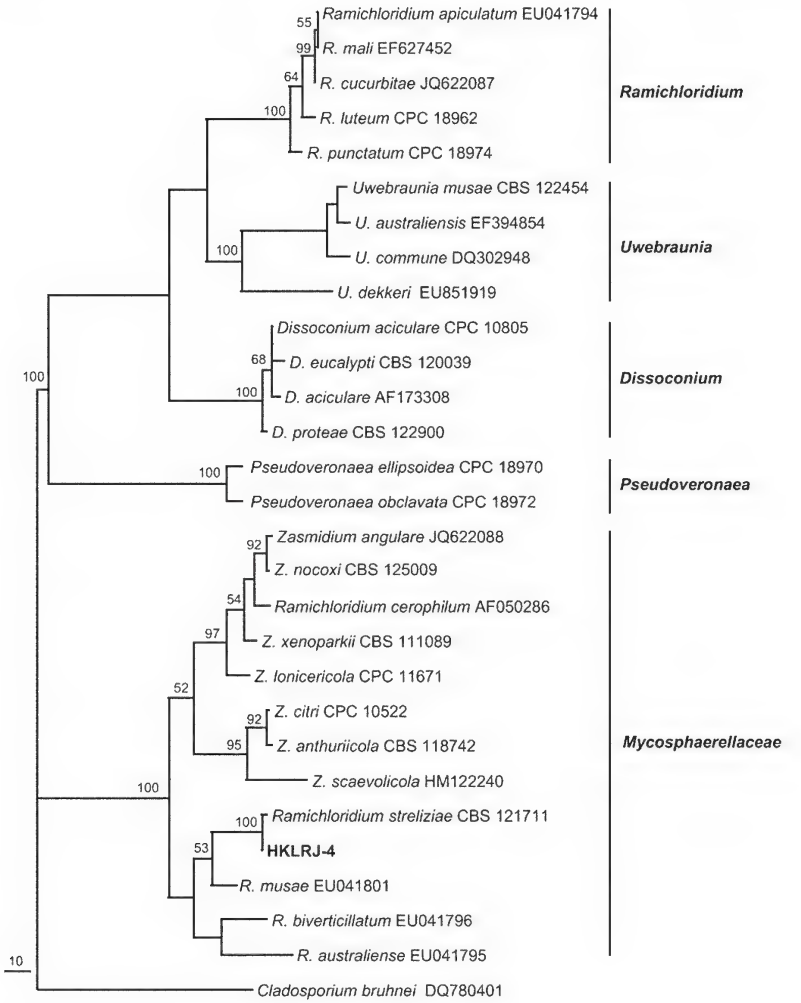

Fig. 1. One of 10 equally parsimonious trees determined from ITS sequences. Bootstrap support values (>50%) based on 1000 replicates are shown at the node. (CI = 0.6611, RI = 0.8657, RC = 0.5723, HI = 0.3389). The scale bar shows 10 changes. The tree is rooted to *Cladosporium bruhnei* and the new sequence is presented in bold.

had a bootstrap value of 100% including twelve species in *Ramichloridium* sensu lato and *Zasmidium* (all in *Mycosphaerellaceae*). Our isolate (HKLRJ-4) grouped with *Ramichloridium strelitziae* (*Mycosphaerellaceae*) with 100% bootstrap support, indicating that they might represent the same species.

Morphology and culture characteristics

Ramichloridium strelitziae Arzanlou, W. Gams & Crous, Stud. Mycol. 58: 74. 2007. FIG. 2

MYCELIUM consisting of smooth, thin-walled, septate, verrucose, pale brown hyphae, 2–3 µm wide. CONIDIOPHORES erect, solitary, arising from creeping aerial hyphae, subhyaline, later becoming pale brown, thick-walled, 1–3(–4)-septate, smooth or verruculose, 2 µm wide, up to 43 µm long. CONIDIOGENOUS cells terminally integrated, (8–)11–38 µm long, subhyaline, later turning pale brown, fertile part as wide as the basal part, sympodially proliferating, forming a straight rachis; SCARS thickened and darkened, approx. 0.5 µm diam. CONIDIA (3.5–)4–5 × (1–)2(–3) µm, solitary, aseptate, subhyaline, smooth or verruculose, oblong, ellipsoidal to clavate, base truncate with unthickened, non-pigmented hilum.

CULTURAL CHARACTERISTICS — COLONY diameter after 14 d on MEA at 24°C reached 6 mm diam with entire margin and compact, raised, dense aerial mycelium, surface olivaceous-grey, reverse olivaceous-black.

HOST CHARACTERISTICS — On stem surface, shiny, black, sclerotium-like bodies, round to oval (120–220 µm diam) with no visible mycelial mat, densely arranged (3–5/mm^2). The flyspeck symptom on *Ravenala madagascariensis* did not physically damage the plants, but greatly reduced their ornamental value.

Based on phylogenetic analysis and morphological characters of the anamorph, we identified isolate HKLRJ-4 as *Ramichloridium strelitziae*.

SPECIMEN EXAMINED: **CHINA. HAINAN PROVINCE**, Haikou City, Jinniuling Park, 20°00′26″N 110°20′31″E, on stems of *Ravenala madagascariensis*, Oct 2011, Li WH (HMUABO HKLRJ-4; GenBank, JX502176).

Discussion

The modern circumscription of *Ramichloridium* is still heterogenous, containing species in two different families (*Dissoconiaceae* and *Mycosphaerellaceae*), and only species clustering in *Capnodiales* were considered to be true *Ramichloridium* (Arzanlou et al. 2007). According to Li et al. (2012) and Arzanlou et al. (2007), *R. apiculatum* (the generic type), *R. cucurbitae*, *R. indicum*, *R. luteum*, *R. mali*, and *R. punctatum* (*Ramichloridium* s. str.) clustered with *Dissoconium* species (all now placed in *Dissoconiaceae*), and other *Ramichloridium* s. lat. species grouped in *Mycosphaerellaceae*. Our strain clustered among the mycosphaerellacean species with *R. strelitziae*.

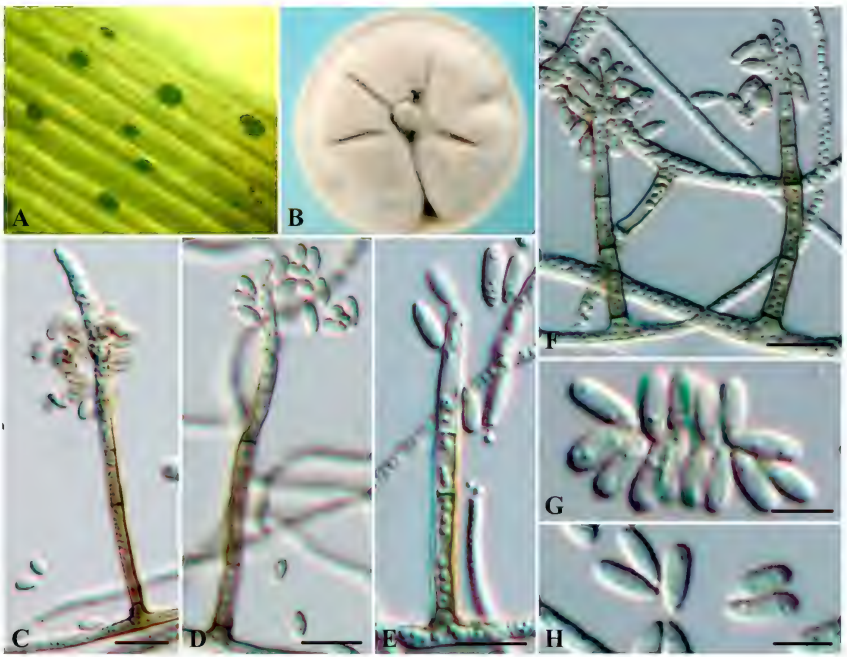

FIG. 2. *Ramichloridium strelitziae* (HKLRJ-4). A. Signs on stem of *Ravenala madagascariensis* with close-up view. B. Colony on MEA after 14 days. C–F. Conidiophores. G–H. Conidia. Bars: C–F = 10μm; G–H = 5μm.

Arzanlou et al. (2007) reported *Ramichloridium strelitziae* from leaves of *Strelitzia nicolai* in South Africa. The species has also been found in bathrooms and washing machines and is able to degrade surfactants, soap, and shampoo (Hamada & Abe 2010). Our results show that *R. strelitziae* may also be involved in the sooty blotch and flyspeck complex.

Acknowledgments

This work was supported by National Natural Science Foundation of China (31170015, 31171797), the 111 Project from Education Ministry of China (B07049), Top Talent Project of Northwest A&F University and the earmarked fund for Modern Agro-industry Technology Research System (nycytx-08-04-04). The authors wish to thank Dr Eric H.C. McKenzie (Landcare Research, Private Bag 92170, Auckland, New Zealand) and Professor Zhongyi Zhang (College of Plant Protection, Yunnan Agricultural University, Kunming, Yunnan, China) for reviewing the manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.45

Volume 124, pp. 45–50 *April–June 2013*

Polycoccum anatolicum sp. nov. on *Lepraria incana* and a key to *Polycoccum* species known from Turkey

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Abstract — *Polycoccum anatolicum* is described as new from the thallus of *Lepraria incana* on the trunk of a *Prunus* sp. in western Turkey and represents the first *Polycoccum* known to occur on *Lepraria*. The new species is typical with its relatively small subglobose ascomata and verruculose relatively large ascospores. It is compared with similar *Polycoccum* species, and a key to *Polycoccum* species known from Turkey is provided.

Key words — *Ascomycota*, lichens, lichenicolous fungi, biodiversity

Introduction

Polycoccum Saut. ex Körb. comprises approximately 50 species of lichenicolous fungi (Halıcı et al. 2007a, 2009; Brackel & Berger 2010; Zhurbenko 2010). The type species of the genus is *P. sauteri* Körb. with lichenicolous habit on *Stereocaulon condensatum* Hoffm. Species are characterized by dark perithecioid ascomata, pseudoparenchymatous exciple, fissitunicate asci with brown one-septate ascospores, and a hamathecium of persistent, branched and anastomosing interascal filaments. Following the genus synopsis by Hawksworth & Diederich (1988), Atienza et al. (2003) recognized 37 species. Brackel & Berger (2010) described three *Polycoccum* species occurring on the lichenised fungus *Placopsis*.

Attention to the lichenicolous fungi of Turkey has increased since Halıcı (2008) published the key to the lichenicolous *Ascomycota*. Of the 181 lichenicolous species known from Turkey (Halıcı et al. 2012), ten *Polycoccum* species have been identified (Halıcı 2008, Halici et al. 2009; Yazıcı et al. 2011). In 2012, the second author (EA) collected an interesting specimen of *Polycoccum* on epiphytic *Lepraria incana* (L.) Ach. s. lat. from Turkey's Mediterranean

Region. After comparison with the described species of the genus, we concluded that the specimen represents a new species, which we describe here. We also provide a key to the eleven *Polycoccum* species now reported from Turkey.

Material & methods

The type of the new species is deposited in Erciyes University Herbarium Kayseri, Turkey (EUH). Sections were prepared by hand and examined in I [Lugol's iodine (MERCK 9261) with (KI) and without (I) pre-treatment with 10% KOH], 10% KOH, and water. Ascospores were measured in water. Ascospore measurements are given as: (min –) X–sd – *X* – X+sd (–max.), where 'min.' and 'max.' are the extreme values, 'X' the arithmetic mean, and 'sd' the corresponding standard deviation. The length/breadth ratio of ascospore is indicated as l/b and given in the same way. The microphotographs were taken with a Leica DFC 420 digital microscope camera with a c-mount interface and with a 5 megapixel CCD. The descriptive notes provided in the key are based on Turkish specimens examined by the first author (MGH), except for *Polycoccum evae,* which is based on the description given by Calatayud & Rico (1995).

Taxonomy

Polycoccum anatolicum Halıcı & E. Akgül, **sp. nov.** FIGURE 1

MYCOBANK MB804154

Differs from *Polycoccum dzieduszyckii* by its 8-spored asci and its *Lepraria* host.

TYPE: Turkey, Isparta, Sütçüler, Yazılı Canyon, 41°48′N 36°31′E, alt. 290 m, on *Lepraria incana* on *Prunus* sp., 18 July 2012, E.Akgül (**Holotype**, EUH (MGH 0.3118)).

ETYMOLOGY: The epithet refers to Anatolia, the Asian part of Turkey, where the type specimen was collected.

Vegetative hyphae hyaline, scant, very narrow, the infection causing no visible necrosis in host, but eventually bleaching the infected parts. Ascomata perithecioid, subglobose, arising singly, in the early stage immersed with only the ostiole area visible, semi-immersed at maturity, 75–90 µm. Ascomatal wall dark reddish brown, darkest in the upper half, 10–15 µm thick, and thickening in the ostiole region; composed of several layers of compressed cells, 10–15 × 6–8 µm, forming a textura angularis. Hamathecium consisting of septate, branched and anastomosing pseudoparaphyses, 1–2 µm thick. Hymenial gelatin K/I–, I–. Asci arising from the lower part of the ascomatal cavity, cylindrical to elongate-clavate, shortly stalked, bitunicate, the apex thickened, 8-spored, (71–)83–91(–103) × (11–)14–18(–21) µm (n = 10); wall I–, endoplasm dextrinoid. Ascospores monostichously arranged in the asci, sole-shaped, apices rounded, some tending to taper towards the rounded ends, 1-septate, not or slightly constricted at the septum, upper cell slightly larger, brown, verruculose, non-halonate, some ascospores multi-guttulate, (25–)26–*28*–29.5(–32) × (8.5–)9–*10*–11(–13) µm (n = 30), l/b = (2.2–)2.52–*2.76*–3(–3.3). Conidiomata not seen.

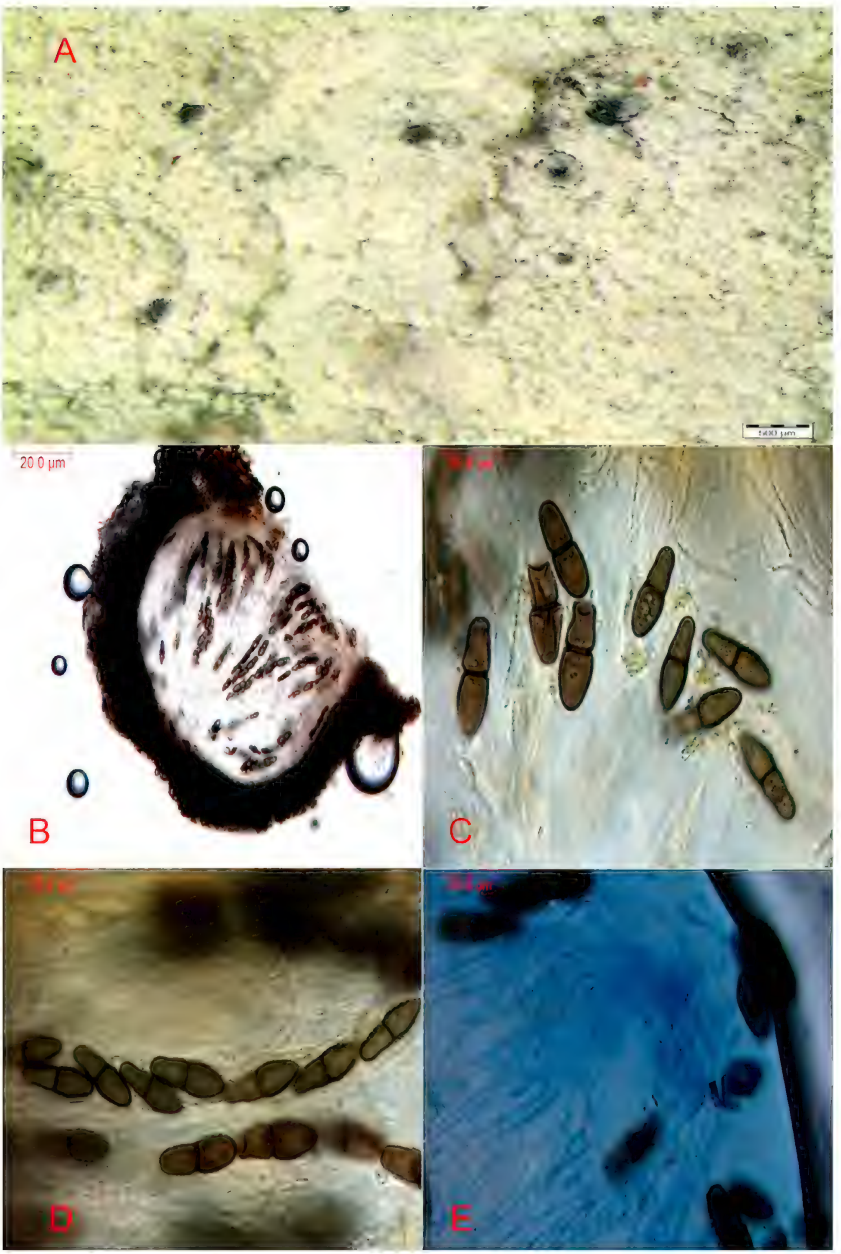

Fig. 1. *Polycoccum anatolicum* (Holotype): A, Habitus; B, Ascoma (in water); C, Ascospores (in water); D, Asci showing monostichously arranged ascospores (in water); E, Anastomosed pseudoparaphyses (in methylene blue).

Ecology & distribution: *Polycoccum anatolicum* is currently known only from the type locality in the Western Mediterranean part of Turkey. The new species was collected on *Lepraria incana* on the trunks of *Prunus* sp. The species seems to be weakly pathogenic as moderate bleaching was observed in the infected parts of the host thallus. As the host lichen has a wide distribution, *P. anatolicum* may also have a wide distribution.

Notes: Most *Polycoccum* species are confined to one host species or genus, and *P. anatolicum* is the only species known to occur on *Lepraria*. It is closest to *P. dzieduszyckii* (Boberski) D. Hawksw., a species known only on *Verrucaria* spp. (and probably other lichens on limestone). *Polycoccum dzieduszyckii* has small ascomata (50–100 µm) and large verruculose ascospores (25–35(–44) × 8–10(–14) µm) (Hawksworth & Diederich 1988, Halıcı et al. 2007b), but clearly differs from *P. anatolicum* in having consistently 2-spored asci and infecting a different host genus.

Polycoccum opulentum (Th. Fr. & Almq.) Arnold, a species known on *Polyblastia intercedens* (Nyl.) Lönnr., *Lecidea plana* (J. Lahm) Nyl., and a sterile lichen on calcareous rocks, has similar sized ascospores (25–28 × 11–14 µm) but thicker pseudoparaphyses (3 µm), larger ascomata (100–170 µm), and hymenial gelatin turning blue in iodine (Hawksworth & Diederich 1988; Atienza et al. 2003). *Polycoccum squamarioides* (Mudd) Arnold, another species known on *Placopsis* spp., has similar sized ascomata (75–100 µm) but narrower ((18–)19–26 × (5.5–)6–7(–8) µm) and smooth-walled ascospores (Hawksworth & Diederich 1988, Brackel & Berger 2010). *Polycoccum evae* Calat. & V.J. Rico is restricted to *Dimelaena oreina* (Ach.) Norman and has larger ascomata (100–200 µm) and shorter verruculose ascospores ((17–)18–23(–25) × (6–)7–10(–11) µm) (Calatayud & Rico 1995). *Polycoccum crassum* Vězda, a species known on *Peltigera* spp., has similar sized verruculose ascospores ((25–)30–32(–36) × 8–10(–11) µm) but much larger ascomata (300–500 µm) and consistently 4-spored asci (Hawksworth & Diederich 1988; Atienza et al. 2003; Halıcı et al. 2012).

A key to the *Polycoccum* species known from Turkey

1. Ascospores <20 µm long 2
1. Ascospores >20 µm long 5
2. Ascospores smooth walled 3
2. Ascospores verruculose, 13–14 × 6 µm, on *Acarospora* spp. ***P. microsticticum***
3. Not causing swellings on the host lichens 4
3. Ascomata c. 200 µm, causing swellings on the host lichens. Ascospores 14–18 × 5–6 µm, on *Peltigera* spp. ..... ***P. peltigerae***

4. Ascomata 100–140 µm. Interascal filaments very thin, c. 1.0 µm. Ascospores (11–)12–14.5(–15.5) × (4.5–)5–6 µm, on *Xanthoria* sp. on coastal rocks ..... ***P. teresum***
4. Ascomata 150–235 µm. Interascal filaments thicker. Ascospores (11–)13.5–15(–16) × 6.5–7 µm, on *Aspicilia cinerea* ..... ***P. aksoyi***
5. Asci >2-spored **6**
5. Asci 2-spored. Ascospores verruculose, 25–28 × 8–11 µm, on endolithic *Verrucaria* spp. on hard calcareous rocks ..... ***P. dzieduszyckii***
6. Asci >4-spored 7
6. Asci 4-spored. Ascomata c. 400 µm. Hymenial gelatin I+ orange-red. Ascospore cells ± equal in size, verruculose, 24–30 × 8–10 µm, on *Peltigera* spp. ..... ***P. crassum***
7. Ascomata >100 µm **8**
7. Ascomata 75–90 µm. Hymenial gelatin I–. Ascospores verruculose, (25–)26–29.5(–32) × (8.5–)9–11(–13) µm, on *Lepraria incana* ... ***P. anatolicum***
8. Hymenial gelatin I– **9**
8. Ascomata c. 220 µm. Hymenial gelatin I+ blue. Ascospores verruculose, 24–27 × 10–13 µm, on endolithic *Verrucaria* spp. ..... ***P. marmoratum***
9. Ascospores verruculose and cells ± equal in size **10**
9. Ascomata 170–200 µm. Ascospores smooth walled and lower cell often attenuate, 22–23 × 10 µm, on *Sporastatia testudinea* ..... ***P. sporastatiae***
10. Ascomata 100–200 µm. Ascospores (17–)18–23(–25) × (6–)7–10(–13) µm, on *Dimelaena oreina* ..... ***P. evae***
10. Ascomata 180–250 µm. Ascospores (25–)28.5–31.5(–34) × (7–)8–9.5 µm, on *Acarospora cervina* ..... ***P. acarosporicola***

Acknowledgements

The manuscript was reviewed by Kerry Knudsen (USA) and Wolfgang von Brackel (Germany). This study was financially supported by the Selçuk University Scientific Research Project Coordinator with the project coded (BAP-12101025).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.51

Volume 124, pp. 51–59 *April–June 2013*

Further additions to the macrolichen mycota of Vietnam

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Abstract — Seven new records of foliose lichens are reported from Chu Yang Sin National Park of Dak Lak province in Vietnam. Morphological, anatomical and chemical characteristics are described for *Heterodermia obscurata*, *Hypotrachyna flavida*, *Leptogium ulvaceum*, *Parmotrema sancti-angelii, Parmeliella brisbanensis*, *Physma byrsaeum*, and *Sticta marginifera*.

Key words — tropical, macrolichens, *Parmeliaceae*, Da Lat Plateau

Introduction

Although larger organisms are well known in the tropics, the fungi and their lichenized counterparts in tropical forests are less known (Coppins & Wolseley 2002). Aptroot & Sipman (1997) observed that the tropical forest lichens represent about one-third to one-half of the world's lichen diversity. Vietnam, being a moderately large country with the monsoon tropical climate (VARCC 2009), provides a range of conditions to support lichen growth (Nguyen et al. 2010, 2011).

Although the lichen mycota of Vietnam is underworked, recently many species have been reported or described. Aptroot & Sparrius (2006) provided the first checklist of Vietnam lichens, which has been followed by new records of macrolichens by Giao (2009), some findings on foliicolous lichens by Nguyen et al. (2010, 2011), and reports of some *Graphidaceae* species by Joshi et al. (2012).

The Korean Lichen Research Institute (KoLRI) has initiated collaboration with Tay Nguyen University in Vietnam, which has resulted in the collection of many lichen specimens, which have been deposited in the herbarium of the KoLRI. This paper reports seven taxa (in five lichen families) from Chu Yang Sin National Park, Vietnam.

Materials & methods

Chu Yang Sin National Park is located in the central highlands of Vietnam in Krong Bong and Lak Districts of Dak Lak Province. This park is situated within the elevations ranging from less than 600 m to 2442 m at the summit of Mount Chu Yang Sin with the area of 58,947 ha. The Park is the largest protected area on the Da Lat Plateau, in the central highlands (Hughes 2010).

The dominant vegetation type in the Park is broadleaved evergreen forest, covering over 65% of the area. This forest is dominated by the vascular plant families *Fagaceae, Lauraceae, Meliaceae,* and *Illiciaceae* with a canopy height often in excess of 35 m. The high altitudinal range and varied topography gives rise to high vascular plant diversity in different forest types. Montane and submontane forests are at >900 m and lowland semi evergreen forests at <900 m (Hughes 2010).

Morphological and anatomical investigations were performed under a binocular dissecting (NIKON SMZ645) and light microscope (Zeiss Scope. A1). Measurements of all the microscopic features were made on hand-cut thallus and apothecial sections. The sections were examined by mounting in water, 10% KOH, and Lugol's iodine solutions. Ascospore measurements were determined in water. Chemical constituents were identified by spot tests and thin layer chromatography performed in solvent systems A (toluene: dioxin:acetic acid 180:45:5) and C (toluene:acetic acid 85:15) (Orange et al. 2010).

Taxonomic descriptions

Heterodermia obscurata (Nyl.) Trevis., Nuovo Gior. Bot. Ital. 1: 114 (1869) Fig. 1a

Thallus foliose, suborbicular, adnate, 2–6 cm across; lobes narrow, up to 1–2 mm wide, flat; upper surface whitish gray, shiny, epruinose, sorediate; soredia granular, labriform to capitate on lateral and terminal lobes; lower cortex absent; lower surface (medulla) felted with yellowish-brown pigment, K+ purple, marginally rhizinate; rhizines black, simple to squarrosely branched; apothecia not seen.

Chemistry — Cortex K+ (yellow), C–, KC–, P+ (pale yellow); medulla K–, C–, KC–, P–. TLC: atranorin, chloroatranorin, zeorin.

Ecology & Distribution — Found on sandy rock in broad-leaved evergreen forest in Chu Yang Sin National Park. According to Moberg & Nash (2001), this species is growing on tree trunk and mossy rocks in open humid conditions. Mostly cosmopolitan and has been reported from many countries in Africa, Asia, Europe, North America, South America, and Oceania (Aptroot & Feijen 2002, Calvelo & Liberatore 2002, Egea 1996, Eliasaro & Adler 1997, Elix & McCarthy 1998, Fryday et al. 2001, Gowan & Brodo 1988, Hafellner & Türk 2001, Knezevic & Mayrhofer 2009, Kurokawa 1960, Llimona & Hladun 2001, López–Figueiras 1986, Osorio 1992, Singh & Sinha 2010, Sipman 1993, Streimann 1986, Tenorio et al. 2002, Wei 1991).

Specimen examined: **VIETNAM. Dak lak province:** Chu Yang Sin National Park, on rock, 12°27′57.0″N 108°20′34.9″E, elev. c. 780 m, 20.04.2012, S.O. Oh, J.S. Hur VN120185 (KoLRI).

Remarks — *Heterodermia obscurata* (*Physciaceae*) is characterized by its robust appearance, labiate soredia, lack of lower cortex, and the rusty-brown pigmented (K+ purple) lower surface or medulla. According to Moberg & Nash (2001), this species is closely related to *H. speciosa* (Wulfen) Trevis., which differs in having a lower cortex and in lacking yellowish pigmentation. It is also easily confused with *H. japonica* (M. Satô) Swinscow & Krog, which has no lower cortex and which is white to violet.

Hypotrachyna flavida (Zahlbr.) Hale, Smithson. Contr. Bot. 25: 37 (1975) Fig. 1b

Thallus adnate to loosely attached, coriaceous, green, 4–6 cm across; lobes sublinear, separate, 2–4 mm wide, margins eciliate; upper surface greenish grey, emaculate, plane to convex, continuous or irregularly cracked on older lobes; medulla white; lower surface moderately rhizinate; rhizines long, sparsely dichotomously branched, often projecting beyond the lobe margins; apothecia adnate, 1–3 mm in diameter, disc brown, flat to concave; Asci clavate, 8 spored, spores colour less, 6–8 μm; pycnidia not seen.

Chemistry — Cortex K–, C–, KC–, P–; medulla K+ (pale yellowish to brown), C–, KC–, P+ (orange-red). TLC: usnic acid, protocetraric acid.

Ecology & Distribution — Found on a sandy rock in broad-leaved evergreen forest in Chu Yang Sin National Park. Reported as saxicolous species at somewhat higher elevations by Hale (1975). This species has previously been reported from Central and South America (Calvelo & Liberatore 2002, Eliasaro & Adler 1997, Hale 1975, Riddle 1920, Tenorio et al. 2002).

Specimen examined: **VIETNAM. Dak lak province:** Chu Yang Sin National Park, on rock, 12°28′12.3″N 108°20′59.9″E, elev. c. 763 m, 20.04.2012, S.O. Oh, J.S. Hur VN120120 (KoLRI).

Remarks — *Hypotrachyna flavida* (*Parmeliaceae*) is characterized by a saxicolous habit and a medulla containing usnic acid and protocetraric acid. According to Hale (1975), *H. flavida* is fairly restricted to northern South America. Externally, this species very closely resembles *H. protoboliviana* (Hale) Hale, which differs in its corticolous habitat and medulla containing barbatic acid.

Leptogium ulvaceum (Pers.) Vain., Ann. Acad. Sci. Fenn., Ser. A, 15(6): 38 (1921) Fig. 1c

Thallus foliose, loosely adnate, 4–8 cm across, bluish gray; lobes rotund, 2–8 mm wide, homiomerous, margins entire, surface smooth to somewhat rough; isidia, phyllidia absent; lower surface smooth, etomentose, pale bluish with sparse tufted rhizines; apothecia laminal, shortly pedicellate, 0.5–2 mm diam., disc flat to concave, red brown, exciple smooth to wrinkled, cells paraplectenchymatous and continuous to below the hypothecium; ascospores

ellipsoid, muriform, 30–40 × 12–18 µm, apices acute to elongate; pycnidia submarginal; conidia 2–3 µm long.

Chemistry — No chemical detected.

Ecology & Distribution — Found on a sandy rock in a shady place in broad-leaved evergreen forest in Chu Yang Sin National Park. This species has previously been reported from Asia, Europe, South America, and Oceania (Awasthi 1988, Elix & McCarthy 1998, Hafellner 1995, Marcelli 1991).

Specimen examined: **VIETNAM. Dak lak province:** Chu Yang Sin National Park, on rock, 12°26′51.9″N 108°20′18.1″E, elev. c. 816 m, 21.04.2012, S.O. Oh, J.S. Hur VN120170 (KoLRI).

Remarks — *Leptogium ulvaceum* (*Collemataceae*) is characterized by smooth upper surface lacking vegetative propagules. This species is morphologically similar to *L. cochleatum* (Dicks.) P.M. Jørg. & P. James, which differs in its periclinally wrinkled thalline exciple.

Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J. Galloway, Flora of Australia, 54: 314 (1992) Fig. 1d

Thallus rosette-forming, orbicular, 5–7 cm across, on a thick, black prothallus projecting 1–2 mm beyond lobe apices, closely appressed to substratum; lobes narrow, 0.6–1 mm wide, discrete at margins, flat; margins entire, somewhat thickened, occasionally ascending; upper surface, slightly maculate, smooth, occasionally striate, grayish when wet, whitish gray when dry, isidiate; isidia delicate, simple to finger-like, to 0.5 mm tall and 0.1 mm thick, laminal and marginal; medulla white to blackish, photobiont *Nostoc*; apothecia, 0.5–2 mm wide, rounded to contorted; exciples thick, thalline, persistent, crenulate-striate, concolorous with thallus; disc flat to concave, red-brown; ascospores simple, ellipsoidal, 10–15 × 8–10 µm; pycnidia not seen

Chemistry — No chemical detected.

Ecology & Distribution — Found on a sandy rock in a shady place in broad-leaved evergreen forest in Chu Yang Sin National Park. According to Jørgensen and Galloway (1992), *Parmeliella brisbanensis* also occurs on tree bark in tropical to subtropical rain forests. This species has been recorded from many countries in the tropics, in Asia, Australia, and Oceania (Elix & McCarthy 1998, Jørgensen & Galloway 1992, Singh & Sinha 2010, Sipman 1993, Wolseley et al. 2002).

Specimen examined: **VIETNAM. Dak lak province:** Chu Yang Sin National Park, on rock, 12°28′04.4″N 108°20′39.0″E, elev. c. 622 m, 20.04.2012, S. O. Oh, J. S. Hur VN120046 (KoLRI).

Remarks: *Parmeliella brisbanensis* (*Pannariaceae*) is characterized by its rosette form, black hypothallus, and finger-like isidiate upper surface. According to Jørgensen & Galloway (1992), this species is the isidiate counterpart of the *P. mariana* (Fr.) P.M. Jørg. & D.J. Galloway complex.

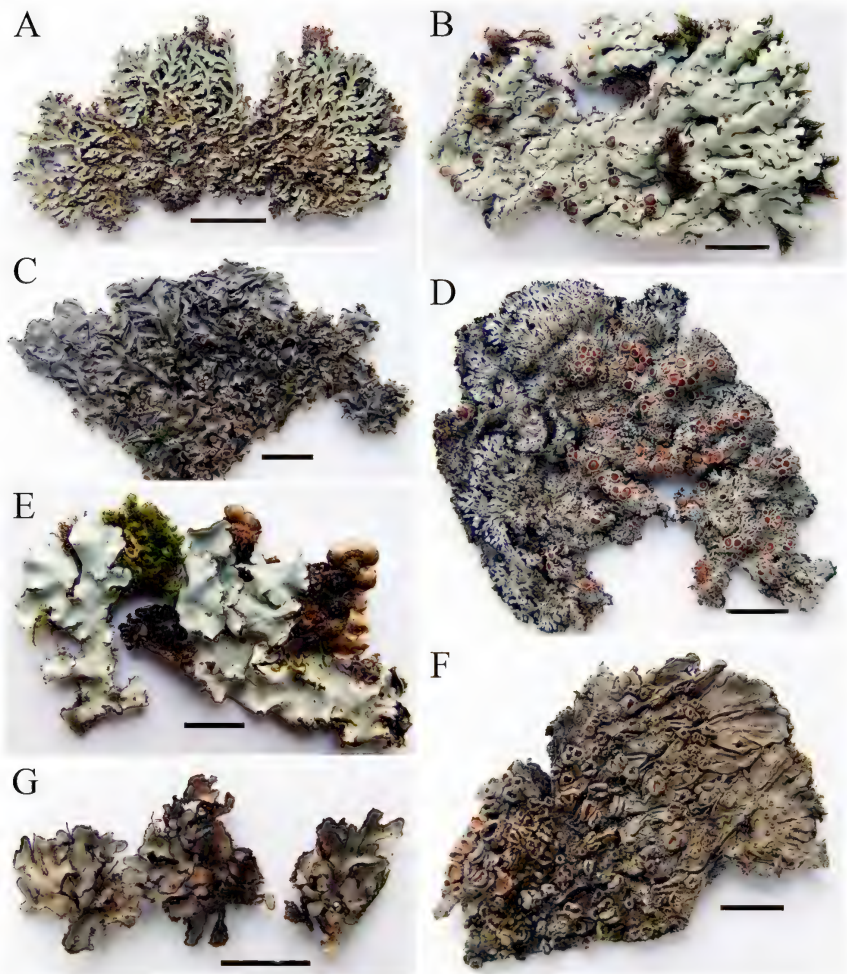

FIGURE 1: External morphology of Vietnamese lichen specimens (Scale bars = 1 cm). A. *Heterodermia obscurata* VN120185 (KoLRI); B. *Hypotrachyna flavida* VN120120 (KoLRI); C. *Leptogium ulvaceum* VN120170 (KoLRI); D. *Parmeliella brisbanensis* VN120046 (KoLRI); E. *Parmotrema sancti-angelii* VN120288 (KoLRI); F. *Physma byrsaeum* VN120107 (KoLRI); G. *Sticta marginifera* VN120053 (KoLRI).

Parmotrema sancti-angelii (Lynge) Hale, Phytologia 28: 339 (1974) FIG. 1E

Thallus loosely attached to the substratum, up to 6 cm across; lobes rotund, 4–10 mm wide; margins crinate, ciliate; cilia numerous, simple, 2–4 mm long; upper surface pale grey, emaculate, smooth, sorediate; soralia marginal, soredia granular; medulla white, yellowish brown pigments below; lower surface, with

relatively narrow, smooth, erhizinate marginal zone; rhizines sparse, simple; apothecia and pycnidia not seen.

Chemistry — Cortex K+ (yellow), C–, KC–, P–; medulla K– C+ (rose), KC+ (red), P–. TLC: atranorin, chloroatranorin, gyrophoric acid and unknown compound.

Ecology & Distribution — Found on a sandy rock in an open place in broad-leaved evergreen forest in Chu Yang Sin National Park. A common and widespread species in the pantropical region (Elix 1994), it has been reported from Asia, Australia, South America, and Oceania (Calvelo & Liberatore 2002, Divakar & Upreti 2005, Elix 1994, Elix & McCarthy 1998, Elix & Schumm 2001, Hale 1974, Kurokawa 1993, Wei 1991, Wolseley et al 2002).

Specimen examined: **VIETNAM. Dak lak province:** Chu Yang Sin National Park, on rock, 12°28′04.4″N 108°20′39.0″E, elev. c. 622 m, 20.04.2012, S.O. Oh, J.S. Hur-VN120288 (KoLRI).

Remarks — *Parmotrema sancti-angelii* (*Parmeliaceae*) is characterized by an emaculate upper surface, sorediate margins, yellowish lower medulla, and brown to black lower surface. This species is very close to *P. indicum* Hale, which differs in having norlobaridone and in lacking pigmentation on the lower medulla.

Physma byrsaeum (Ach.) Tuck., Syn. N. Amer. Lich. 1: 115 (1882) Fig. 1f

Thallus foliose, rosulate, adnate, 6–8 cm across, 200–350 µm thick; lobes radiating, oblong 1–5 mm wide; margins entire, thickened, re-curved at the lobes end; upper surface ridged, somewhat wrinkled, brownish black, lacking isidia; cortex thin, photobiont *Nostoc*; lower surface pale brown, rhizinate; rhizines of interwoven hyphae forming a cushion-like indumentum, whitish to black; ascomata apothecial, abundant, laminal, sessile, 1–4 mm wide; disc concave to plane, reddish brown; thalline exciple thick, wrinkled, lobed, concolorous with thallus; ascospores simple, ellipsoidal, 12–15 × 10–12 µm; episporium, 2–3 µm thick; pycnidia laminal, conidia 2–3 µm long.

Chemistry — No chemicals detected.

Ecology & Distribution — Found on sandy rock in a shady place in broad-leaved evergreen forest in Chu Yang Sin National Park. *Physma byrsaeum* has been reported from many countries in the paleotropics, including Asia, Australia, Central America, and Oceania (Aptroot & Seaward 1999, Aptroot & Sipman 1991, Elix & McCarthy 1998, Sipman 1993, Tenorio et al. 2002, Verdon 1992, Wolseley et al. 2002).

Specimen examined: **VIETNAM. Dak lak province:** Chu Yang Sin National Park, on rock, 12°28′12.3″N 108°20′59.9″E, elev. c. 763 m, 20.04.2012, S.O. Oh, J.S. Hur VN120107 (KoLRI).

Remarks — *Physma byrsaeum* (*Collemataceae*) is characterized by a well-developed cortical layer and wrinkled thalline apothecial exciple. This species

is morphologically similar to *P. pseudoisidiatum* Aptroot & Sipman, which is isidiate.

Sticta marginifera Mont., Annls Sci. Nat., Bot., Sér. 2 18: 265 (1842) FIG. 1G

Thallus palmate to irregularly branched in clumps, 1–2 cm across, arising from a rooted holdfast; lobes flabellate to irregularly dichotomously branching, 1–3 mm wide, proliferating round to oblong lobules attached to primary lobes by thin terete stalks; margins entire or irregularly notched, thickened below; upper surface grayish glaucous when dry, dark blue-black when wet, thin, papery, smooth, rather fragile, isidiate, phyllidiate and minutely maculate; isidia marginal, sometimes on the upper surface, styliform to coralloid, to 0.5 mm tall; phyllidia marginal, developing from isidia, to 1 mm tall, irregularly lobed; photobiont *Nostoc*; lower surface white to pale at margins, brown centrally, minutely pubescent; cyphellae scattered, round, to 0.5 mm diam.; pit membrane white; apothecia not seen.

CHEMISTRY — No chemicals detected.

ECOLOGY & DISTRIBUTION — Found on sandy rock in a shady place in broad-leaved evergreen forest in Chu Yang Sin National Park. According to Galloway (2001), *Sticta marginifera* prefers humid, deeply shaded habitats in montane rain forests on different substrates. This species has been recorded from Asia, Australia, South America, and Oceania (Awasthi 2007, Calvelo & Liberatore 2002, Elix & McCarthy 1998, Galloway 2001, Streimann 1986).

SPECIMEN EXAMINED: **VIETNAM. DAK LAK PROVINCE:** Chu Yang Sin National Park, on rock, 12°28′04.4″N 108°20′39.0″E, elev. c. 622 m, 20.04.2012, S.O. Oh, J.S. Hur VN120053 (KoLRI).

REMARKS — *Sticta marginifera* (*Lobariaceae*) is characterized by the presence of secondary lobules and the coralloid isidia. According to Galloway (2001), the similar *S. brevipes* (Müll. Arg.) Zahlbr. also has similar flabellate lobes with thickened and revolute apices but does not produce secondary lobules.

Acknowledgements

This work was supported by a grant from the National Research Foundation of Korea (#2011–0031494) and the Korea National Research Resource Center Program. Authors are grateful to Ms. Pat Wolseley and Dr. Sanjeeva Nayaka for their valuable comments on manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.61

Volume 124, pp. 61–68 *April–June 2013*

Inonotus niveomarginatus and *I. tenuissimus* spp. nov. (*Hymenochaetales*), resupinate species from tropical China

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Abstract — Two new species are described from tropical Yunnan, southwestern China. *Inonotus niveomarginatus* is characterized by resupinate basidiocarps with distinct white margins, a monomitic hyphal structure, absence of hymenial and hyphoid setae, and yellowish, thick-walled, ovoid to subglobose basidiospores. *Inonotus tenuissimus* can be distinguished from other *Inonotus* species by the combination of resupinate habit, a mono- to dimitic hyphal structure, absence of the hymenial and hyphoid setae, and yellowish thick-walled ellipsoid basidiospores.

Key words — *Hymenochaetaceae*, polypore, taxonomy, wood-inhabiting fungi

Introduction

Yunnan Province in southwestern China is one of the biodiversity "hotspots" in the world and rich with many vascular plants and fungi. Many wood-decaying fungi have been described from this province (Chen & Cui 2012; Cui et al. 2009; Dai 2010, 2011; Dai et al. 2002, 2009; He & Dai 2012; Li & Cui 2010, 2013; Yuan & Dai 2008; Zhao et al. 2013). During a systematic survey of *Inonotus* P. Karst. species in southwestern China, two resupinate species were found that lacked hymenial and hyphoid setae and possessed yellowish thick-walled ovoid to subglobose or ellipsoid basidiospores. These prominent characters distinguish the two species from all other known *Inonotus* species, and they are described here as new.

Materials & methods

The studied specimens were deposited at the herbarium of Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic procedures were follows as in Cui & Decock (2013). Sections were studied at magnifications up to 1000× using a Nikon Eclipse 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and illustrations were made

from the slide preparations stained with Cotton Blue and Melzer's reagents. Spores were measured from sections cut from the tubes. To present the spore size variation, the 5% of measurements excluded from each end of the range are given in the parentheses; spine lengths are not included in the basidiospore measurements. Abbreviations include IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, and n = number of spores measured from the given number of specimens. Special color terms follow Petersen (1996).

Taxonomy

Inonotus niveomarginatus H.Y. Yu, C.L. Zhao & Y.C. Dai, **sp. nov.** FIGS 1–2

MycoBank MB805435

Differs from other *Inonotus* species by its resupinate growth habit, absence of hymenial and hyphoid setae, and yellowish, thick-walled, ovoid to subglobose basidiospores.

Type: China, Yunnan Province, Xishuangbanna, Jinghong County, Sanchahe Nature Reserve, on fallen angiosperm trunk, 7.VI.2011, Dai 12318 (**holotype**, BJFC).

Etymology: *niveomarginatus* (Lat.): refers to the white margin of the basidiocarp.

Fruitbody — Basidiocarps annual, resupinate, cushion-shaped, adnate, without odor or taste when fresh, becoming corky upon drying, ≤4 cm long, 2.5 cm wide, 4 mm thick at centre. Pore surface deep olive and margin white when fresh, dark brown to yellowish brown upon drying; pores circular, 6–8 per mm; dissepiments thin, entire. Subiculum brown, corky, ≤1 mm thick. Tubes dull brown to blackish brown, corky, ≤3 mm long.

Hyphal structure — Hyphal system monomitic; generative hyphae simple septate; tissues darkening in KOH.

Subiculum — Generative hyphae yellow, thin- to thick-walled with a wide lumen, frequently septate, very rarely branched, more or less straight, interwoven, 3–4.5 µm in diam.

Tubes — Generative hyphae yellowish, thin- to thick-walled, frequently branched and septate, more or less straight, subparallel along the tubes, 2.5–3.5 µm in diam. Basidia barrel-shaped, with four sterigmata and a basal simple septum, 13–16 × 6–8.5 µm; basidioles in shape similar to basidia, but slightly smaller than basidia.

Spores — Basidiospores ovoid to subglobose, yellowish, thick-walled, smooth, occasionally bearing a guttule, IKI–, CB–, (4.5–)4.9–5.7(–6) × (4.2–)4.5–5.2(–5.5) µm, L = 5.35 µm, W = 4.95 µm, Q = 1.06 (n = 30/1).

Remarks — Four other *Inonotus* species — *I. costaricensis* Ryvarden, *I. rigidus* B.K. Cui & Y.C. Dai, *I. truncatisporus* Corner, and *I. venezuelicus* Ryvarden — also are characterized by resupinate basidiocarps, thick-walled colored spores,

FIGURE 1. *Inonotus niveomarginatus* (holotype). Basidiocarp.

and a lack of both hymenial and hyphoid setae (Corner 1991; Cui et al. 2011; Ryvarden 1987, 2005). *Inonotus costaricensis* differs from *I. niveomarginatus* by having globose and longer basidiospores (5.5–6.5 μm long; Ryvarden 1987). *Inonotus rigidus* differs from *I. niveomarginatus* by its ellipsoid and smaller

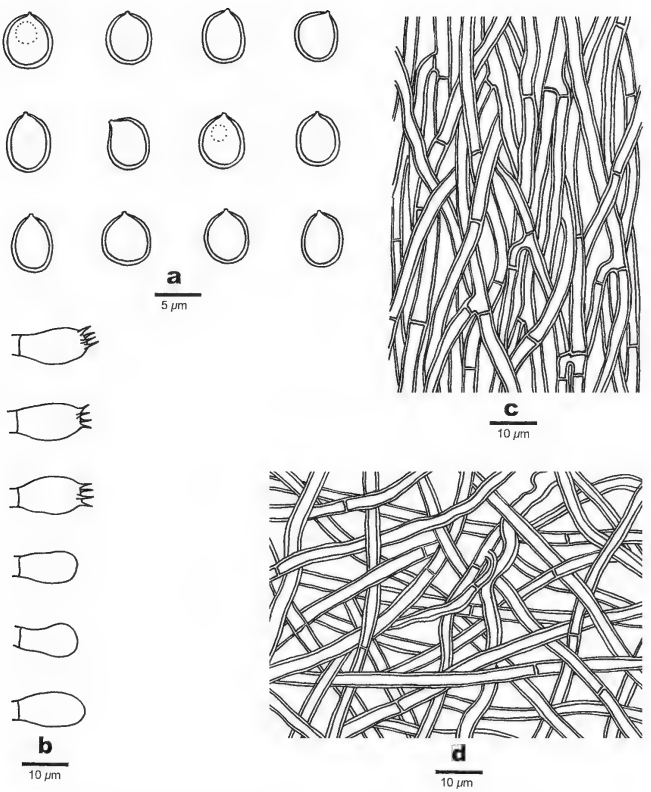

Figure 2. *Inonotus niveomarginatus* (holotype) microscopic structures. a: Basidiospores. b: Basidia and basidioles. c: Hyphae from trama. d: Hyphae from subiculum.

spores (3.9–4.5 × 2.9–3.7 μm; Cui et al. 2011). *Inonotus truncatisporus* is distinguished from *I. niveomarginatus* by its larger pores (4–6 per mm) and truncate basidiospores (Corner 1991). *Inonotus venezuelicus* has larger pores (3–4 per mm), ellipsoid basidiospores (5–6 × 4.5–5 μm), and has been found only in South America (Ryvarden 1987).

Inonotus tenuissimus H.Y. Yu, C.L. Zhao & Y.C. Dai, **sp. nov.** Figs 3–4

MycoBank MB805434

Differs from other *Inonotus* species by its resupinate basidiocarps, a mono- to dimitic hyphal structure, absence of hymenial and hyphoid setae, and yellowish, thick-walled, ellipsoid basidiospores.

Type: China, Yunnan Province, Puer County, Laiyanghe Forest Park, on fallen angiosperm trunk, 6.VI.2011, Dai 12365 (**holotype**, BJFC).

Etymology: *tenuissimus* (Lat.): refers to the very thin subiculum.

FIGURE 3. *Inonotus tenuissimus* (holotype). Basidiocarp.

FRUITBODY — Basidiocarps annual, resupinate, adnate, becoming corky upon drying, ≤15 cm long, 7 cm wide, 1.5 mm thick at centre. Pore surface vinaceous grey to greyish brown when fresh, pale brown upon drying; pores angular, 3–4 per mm; dissepiments thin, entire. Subiculum brown, corky, up to 0.2 mm thick. Tubes dull brown, corky, ≤1.3 mm long.

HYPHAL STRUCTURE — Hyphal system mono- to dimitic; generative hyphae, simple septate; tissues darkening in KOH.

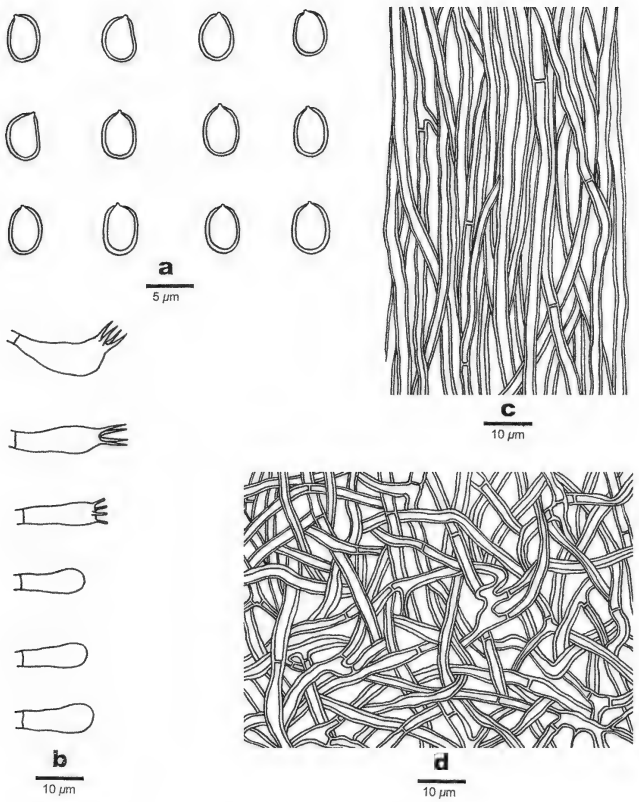

Figure 4. *Inonotus tenuissimus* (holotype) microscopic structures.
a: Basidiospores. b: Basidia and basidioles. c: Hyphae from trama.
d: Hyphae from subiculum.

Subiculum — Generative hyphae yellow, thick-walled, frequently septate, more or less straight, interwoven, 2.5–4.5 μm in diam. Some skeletal-like hyphae similar to generative hyphae, rarely septate.

Tubes — Generative hyphae yellowish, thick-walled, occasionally branched, rarely septate, subparallel along the tubes, 3–4 μm in diam. Some skeletal-like hyphae similar to generative hyphae, rarely septate. Basidia clavate to barrel-shaped, with four sterigmata and a basal simple septum, 12–18 × 4.5–6.5 μm; basidioles barrel-shaped, smaller than basidia.

Spores — Basidiospores ellipsoid, yellowish, thick-walled, smooth, IKI–, CB–, (4–)4.3–5(–5.2) × (3–)3.2–4(–4.2) μm, L = 4.8 μm, W = 3.6 μm, Q = 1.31–1.38 (n = 90/3).

Additional specimens examined: **CHINA. Yunnan Province**, Puer County, Laiyanghe Forest Park, on fallen angiosperm branch, 6.VI.2011, Dai 12245 & Dai 12255 (BJFC); 9.VI.2011, Dai 12330 (BJFC).

Remarks — *Inonotus tenuissimus* resembles *I. venezuelicus* in its resupinate basidiocarps, similar pores, thick-walled and colored spores, and absence of both hymenial and hyphoid setae. However, *I. venezuelicus* has greyish pores when dry, a distinctly monomitic hyphal structure, larger and ellipsoid to ovoid basidiospores (5–6 × 4.5–5 μm), and has been found only in South America (Ryvarden 2005).

Acknowledgments

We express our gratitude to Dr. Li-Wei Zhou (IFP, China) and Mr. Jaya Seelan Sathiya Seelan (Clark University, USA) who reviewed the manuscript. The research was financed by the National Natural Science Foundation of China (Nos. 31070022, 30910103907).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.69

Volume 124, pp. 69–72 *April–June 2013*

Arenariomyces truncatellus sp. nov., an ascomycete on driftwood from the north coast of Zealand, Denmark

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Abstract — Incubated driftwood from the north coast of Zealand, Denmark, yielded a new species, *Arenariomyces truncatellus,* belonging to the *Halosphaeriaceae, Ascomycota*. The species is characterized by globose, spiny ascomata formed on wood with asci deliquescing before spore maturity and 1-septate ascospores with three delicate appendages at each spore end. The species is compared with *A. majusculus, A. parvulus, A. trifurcatus,* and *A. triseptatus*. A key to the species is provided.

Key words — higher marine fungi, *Microascales*, taxonomy

Introduction

Investigations into the incidence and distribution of higher marine fungi colonizing wood along the Danish coasts were initiated by Höhnk (1955) and Kohlmeyer (1968). Since then more data have been accumulated and published in "A check list of higher marine fungi on wood from Danish coasts" (Koch & Petersen 1996) and further in Petersen (1997), Petersen & Koch (1997a,b), and Koch et al. (2007). Also added (Koch unpublished) are *Corollospora angusta* Nakagiri & Tokura, *C. californica* Kohlm. & Volkm.-Kohlm., and *C. pseudopulchella* Nakagiri & Tokura. In this article, a new species of *Arenariomyces* Höhnk (*Halosphaeriaceae*) is described from the north coast of Zealand, Denmark.

Material & methods

Driftwood entrapped among stones and wood from the intertidal zone was collected at Tisvildeleje on the north coast of Zealand, Denmark, in January 2008. Salt content was around 10–20‰. The wood was subsequently incubated at 16–19°C and examined periodically for fruiting bodies. From the first formed *A. truncatellus* ascomata, ascospores were used for inoculation of sterilized pieces of driftwood, on which ascomata developed over a 4–8 month incubation period at 16–19°C ascomata developed.

Arenariomyces truncatellus Jørg. Koch, **sp. nov.** FIGS 1–5

MycoBank 50644

Differs from *Arenariomyces majusculus* by its smaller ascospores with two cells often conspicuously different in size and with short delicate appendages.

TYPE: Denmark, North Zealand, Tisvildeleje (UTM 691000E 6217000N), from driftwood (*Picea* sp.), Jan. 2008, Jørg. Koch 998, incubated nine months in humid chamber (**Holotype**, CP [piece of conifer wood + slides 1–4]; **isotype**, CP [remainder of collection Jørg. Koch 998]). [The original material also contained *Dictyosporium pelagicum* (Linder) G.C. Hughes ex E.B.G. Jones and *Halosphaeriopsis mediosetigera* (Cribb & W. Cribb) T.W. Johnson.]

ETYMOLOGY: From the Latin *truncatellus* = somewhat truncate, referring to the slightly flattened ends of the ascospores, when appendages are not unfolded.

ASCOMATA on wood (rarely seated with subicula on grains of sand) measured in situ 78–131–156 µm (n = 19) in diam. Globose-subglobose with the basal part immersed in the outermost layer of the substrate and anchored with brown tortuous hyphae, often in tufts. Ascomata gregarious, black, shiny when young, carbonaceous, brittle, covered with scattered brownish black spines, slightly curved, up to 16 µm long and 1.5–2 µm wide, but sometimes with a swollen base 4–8 µm wide and 4 µm high. Immature ascomata from 20 µm in diam. are covered with spines up to 8 µm long. Neither necks nor ostioles were observed. PERIDIUM membranous, single layered, composed of one layer of flat, on the outside domed, thick-walled cells forming a textura angularis with edges 3–4–6 µm, brownish black in transparent light. SUBICULUM composed of hyaline, epidermoid cells merging into the thin-walled pseudoparenchyma, globose or ellipsoidal as free cells (10–20–26 µm in diam.), with scattered cytoplasm and small oil globules filling the centre of young ascomata. In some cells a few cytoplasmic strands stretching towards the cell wall indicate pit connections to neighbouring cells.

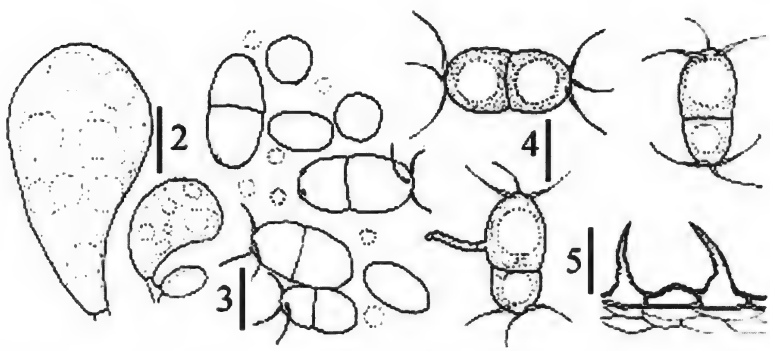
ASCI eight-spored, pyriform, 36–56 × 20–32 µm, thin-walled, unitunicate, deliquescing before maturity of the ascospores, developing from the base of the ascocarp. No catenophyses or paraphyses. ASCOSPORES 18–21.4–24 × 8–10.2–12 µm (n = 66) measured in seawater (12%), cylindrical, 1-septate, more or less constricted at the septa, often one cell is shorter and narrower than the other, hyaline, with three subterminal-terminal appendages at each end. APPENDAGES attenuate, straight or curved, delicate, up to 12 µm long, about 1 µm wide at the base, expanded at right angles to the axis, 120° between each appendage. When not unfolded the spore ends seem slightly flattened.

SUBSTRATE: Driftwood (conifer wood) between stones (intertidal zone).

Discussion

Jones et al. (2009) recognized the four species *A. majusculus* Kohlm. & Volkm.-Kohlm., *A. parvulus* Jørg. Koch, *A. trifurcatus* Höhnk, and *A. triseptatus*

FIG. 1. *Arenariomyces truncatellus.* Group of ascomata formed on sterilized driftwood inoculated with ascospores. Scale bar = 160 µm.



FIGS 2–5. *Arenariomyces truncatellus.* 2. Young asci. 3. Different stages of immature ascospores forming an eight-spored cluster indicating an early deliquescing of the ascus wall. 4. Three ascospores, one germinated after 72 hours in sea water (12%). 5. Part of the peridium with spines and underlying epidermoid flattened cells. Scale bars: 2–4 = 10 µm; 5 = 14 µm.

Kohlm. in the genus *Arenariomyces*. The latest, *A. truncatellus*, is different from these with respect to ascospore dimensions, the uneven sized spore cells, and the short and delicate structure of the appendages. *A. truncatellus* and *A. majusculus* ascomata are formed in the outermost part of the wood substrate and bearing spines in contrast to *A. parvulus*, *A. trifurcatus*, and *A. triseptatus*. However, the stability of ascomatal characters in the genus is questioned (on wood / on hard surfaces, spines / no spines). For example, *A. trifurcatus* ascomata are sometimes seen with a few spines and also directly on wood (Koch unpublished). Further observations of the rare *Arenariomyces* species could perhaps reveal a similar variability. But general similarities such as the thin-walled pseudoparenchyma of the young ascomata, unitunicate asci that deliquesce early before ascospore maturation, together with the three polar appendages point to *A. truncatellus*

as morphologically well accommodated as a new species in *Arenariomyces*. It is recognized that examination of *A. truncatellus* in the electron microscope, especially in regard to appendage development, and at the molecular level is needed to establish its relationship to *A. trifurcatus*, the type species.

Key to *Arenariomyces* species

1a. Ascomata mainly on wood **2**
1b. Ascomata mainly on hard surfaces..... **3**
2a. Ascospores 33 × 11.5 µm (av.)..... ***A. majusculus***
2b. Ascospores 21.4 × 10.2 µm (av.) ..... ***A. truncatellus***
3a. Ascospores 3-septate..... ***A. triseptatus***
3b. Ascospores 1-septate **4**
4a. Ascospores 16–25 × 3–6 µm ..... ***A. parvulus***
4b. Ascospores 28–32 × 9–11 µm ..... ***A. trifurcatus***

Acknowledgments

I am grateful to Iben M. Thomsen and Flemming Rune, both at Forest and Landscape Denmark (Faculty of Life Sciences, University of Copenhagen) for photographic work, and to Iben M. Thomsen for preparing the manuscript for printing. Also I am grateful to Dr. E.B. Gareth Jones and Dr. K.L Pang for reviewing the manuscript and giving valuable comments.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.73

Volume 124, pp. 73–85 *April–June 2013*

New ascomycete records from Guatemala

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Abstract — Thirty-two species of ascomycetes were previously recorded from Guatemala. This paper describes 12 newly recorded species, representing five orders and twelve genera. Three previously reported species are redescribed because there are few data in the Guatemalan literature.

Key words — *Helotiales, Hypocreales, Orbiliales, Pezizales, Xylariales*, Central America

Introduction

Guatemala is a country of >108,000 km^2. The name Quauhtemallan (Guatemala) means "land of trees," referring to the original wide extent and diversity of forests. Guatemala has been categorized as biologically megadiverse (Tolisano & López 2010), but little is known about the fungal diversity of this country and adjacent areas. There have been few studies on fungal diversity, and the knowledge of the ascomycetes is poor; only 33 ascomycetes species have been reported in previous studies (Table 1). The present study is part of a project that began in 2007 to document the ascomycete diversity of Guatemala.

Materials & methods

The study was based on a bibliographic revision and additional material collected mainly from oak forests and cloud forests of the west of Guatemala. The following literature was used for identifications: Abbot & Currah (1997); Breitenbach & Kränzlin (1984); Dennis (1954; 1963; 1970; 1978), Glawe & Rogers (1984), Haines & Dumont (1984); Hsieh et al. (2005); Ju & Rogers (1996); Medel (2002) Medel & Calonge, (2004); Méndez-Mayboca et al. (2007); Ramamurthi et al. (1957); San Martín et al. (1998), and

Table 1. Ascomycete species recorded from Guatemala.
(New records in bold font.)

Helotiales	
Bisporella citrina	This paper
Chlorociboria aeruginascens (Nyl.) Kanouse ex C.S. Ramamurthi et al.	Flores et al. (2012)
C. aeruginosa	This paper
Lachnum abnorme	This paper
L. brasiliense	Haines & Dumont (1984); this paper
L. cyphelloides	This paper
L. virgineum	This paper
Leotia lubrica (Scop.) Pers.	Sommerkamp & Guzmán 1990
Trichoglossum farlowii (Cooke) E.J. Durand	Flores et al. (2002, 2012)
Hypocreales	
Cordyceps melolonthae (Tul. & C. Tul.) Sacc.	Flores et al. (2012)
C. militaris (L.) Fr.	Flores et al. (2012)
Hypomyces hyalinus (Schwein.) Tul. & C. Tul.	Sommerkamp & Guzmán (1990)
H. lactifluorum (Schwein.) Tul. & C. Tul.	Bran et al. (2003)
Ophiocordyceps gracilis	This paper
Orbiliales	
Hyalorbilia inflatula	This paper
Orbilia juruensis	This paper
Pezizales	
Aleuria aurantia (Pers.) Fuckel	Sommerkamp & Guzmán (1990)
Cookeina sulcipes (Berk.) Kuntze	Sommerkamp & Guzmán (1990)
Gyromitra infula	Bran et al. (2003): this paper
Helvella acetabulum	This paper
H. crispa (Scop.) Fr.	Bran et al. (2003)
H. elastica Bull.	Sommerkamp & Guzmán (1990)
H. lacunosa Afzel.	Bran et al. (2003); Sommerkamp & Guzmán (1990)
H. macropus (Pers.) P. Karst.	Bran et al. (2003); Sommerkamp & Guzmán (1990)
Melastiza chateri (W.G. Sm.) Boud.	Flores et al. (2002)
Morchella costata (Vent.) Pers.	Sommerkamp & Guzmán, (1990)
M. elata Fr.	Bran et al. (2003)
M. esculenta (L.) Pers.	Sommerkamp & Guzmán (1990); Bran et al. (2003)
M. guatemalensis Guzmán et al.	Guzmán et al. (1985)
Otidea onotica (Pers.) Fuckel	Flores et al. (2002)
Phillipsia guatemalensis Paden	Paden 1977
Pithya cupressina (Batsch) Fuckel	Sommerkamp & Guzmán (1990)
Scutellinia scutellata (L.) Lambotte	Sommerkamp & Guzmán (1990)
Wynnea americana Thaxt.	Flores et al (2002; 2012)
Xylariales	
Annulohypoxylon thouarsianum	Sharp (1948), Flores et al. (2012); this paper
Daldinia concentrica	This paper
D. fissa Lloyd	Morales et al. (2006)
D. vernicosa Ces. & De Not.	Bran et al. (2003)
Diatrypella pulvinata	This paper
Phylacia poculiformis (Mont.) Mont.	Sommerkamp & Guzmán (1990)
Poronia pileiformis	This paper
Xylaria cubensis (Mont.) Fr.	Sharp (1948)
X. multiplex (Kunze) Fr.	Sommerkamp & Guzmán (1990)
X. polymorpha (Pers.) Grev.	Flores et al. (2012)

Spooner (1987). All material studied was deposited in the mycological collection at XAL herbarium (Instituto de Ecología, A.C., Veracruz, Mexico) and duplicate specimens were deposited in mycological collection MICG (Universidad de San Carlos, Facultad de Ciencias Químicas, Guatemala, Guatemala). Microscopic features were examined in KOH 5% and Melzer's iodine reagent, and at least 20 measurements of ascospores were made per collection. Photographs were taken with a Sony Cyber Shot Camera, and some photographs of spores were cleared of spots, stains and bubbles using Photoshop CS5 software.

Helotiales

Bisporella citrina (Batsch) Korf & S.E. Carp., Mycotaxon 1: 58 (1974) FIG. 1

Apothecia discoid to cupulate shaped, attached to the substrate by a short stalk. Disc 1 mm diameter. Stalk 0.6 mm long, cylindrical. Hymenium bright yellow and smooth outer surface. Asci cylindrical, 57–80 × 5–7 µm, small pore blue in Melzer's reagent. Ascospores elliptical, smooth hyaline, with 2 guttules, with a single septum when mature, 7–10 × 2–3 µm. Paraphyses filiform with rounded tips, 1–1.5 µm thick, hyaline, thin walled.

HABITAT — Gregarious, lignicolous; in cloud forest at 1500 m.

MATERIAL EXAMINED — **GUATEMALA. CHIMALTENANGO DEPARTMENT**, San Andres Itzapa Municipality, Aldea La Hierbabuena, 27 June 2007, Medel 1451 (XAL).

The bright yellow apothecia and ascospore size are diagnostic for this species. *Bisporella citrina* is widely distributed (Dennis 1970, 1978; Breitenbach & Kränzlin 1984; Medel & Calonge 2004; Méndez-Mayboca et al. 2007). This represents a new record for Guatemala.

Chlorociboria aeruginosa (Oeder) Seaver ex C.S. Ramamurthi, Korf & L.R. Batra, Mycologia 49: 859 (1958) ["1957"] FIG. 2

Apothecia globose to discoid shaped, irregularly distorted, 12 mm diameter, attached to the substrate by a short central stipe; hymenium smooth, blue-green. Stalk cylindrical, 0.5 mm long. Asci cylindrical, 8-spored, (57–) 70–80 × 5–6 µm, small blue pore in Melzer's reagent. Ascospores irregularly fusiform, smooth, hyaline, with 2 guttules, 9–15 × 2–3 µm. Paraphyses with rounded tips, 1–1.5 µm thick.

HABITAT — Gregarious, lignicolous; in *Cupressus* forest at 1500 m.

MATERIAL EXAMINED — **GUATEMALA. GUATEMALA DEPARTMENT**, Universidad de San Carlos de Guatemala, University Campus, Parque Ecológico Las Ardillas, 28 June 2007, Medel 1495 (XAL); **CHIMALTENANGO DEPARTMENT**, San Andrés Itzapa Municipality, Aldea la Hierbabuena, 27 June 2007, Medel 1446 (XAL, MICG).

According to Dixon (1975) this species is close to *C. aeruginascens,* which also has the same green color but which differs in smaller ascospores (6–10 × 1.5–2 µm). *Chlorociboria aeruginosa* is distributed in North, South, and Central

America, China, India, Japan, and the Philippines (Dixon 1975) and Venezuela (Mardones-Hidalgo & Iturriaga 2011). Despite being a widely distributed species, in Guatemala the species was not known.

Lachnum abnorme (Mont.) J.H. Haines & Dumont, Mycotaxon 19: 10 (1984)

FIGS 3–4

Apothecia discoid, with white hairs, stipe very small and sometimes almost sessile, 1 mm wide × 0.5 mm tall, hymenium light to dark yellow. Hairs subcylindrical with blunt apex, with coarse granules, hyaline, with 2-3 septa, thin walled, (40–)60–88 × (3–)3.5–4 µm. Asci cylindrical, 8-spored (75–)87–94 × 7–8 µm, small blue pore in Melzer's reagent. Ascospores straight to s-shaped, hyaline, (38–)40–57 × 2 µm. Paraphyses lanceolate, (77–)94–102(–110) × 3–3.5 µm, hyaline, 3–4-septate.

HABITAT — Gregarious, lignicolous; in *Cupressus* forest at 1500 m.

MATERIAL EXAMINED — **GUATEMALA. GUATEMALA DEPARTMENT**, Universidad de San Carlos de Guatemala, University City, Parque Ecológico Las Ardillas, 28 June 2007, Medel 1486 (XAL).

The studied specimen fitted well with the descriptions by Dennis (1963), Haines & Dumont (1984), and Spooner (1987). *Lachnum abnorme* is widely distributed in tropical regions of the world: Australia, New Zealand, Chile, India, Central America, and Costa Rica (Haines & Dumont 1984); Panama (Piepenbring 2006); and Venezuela (Mardones-Hidalgo & Iturriaga 2011). This is the first record to Guatemala.

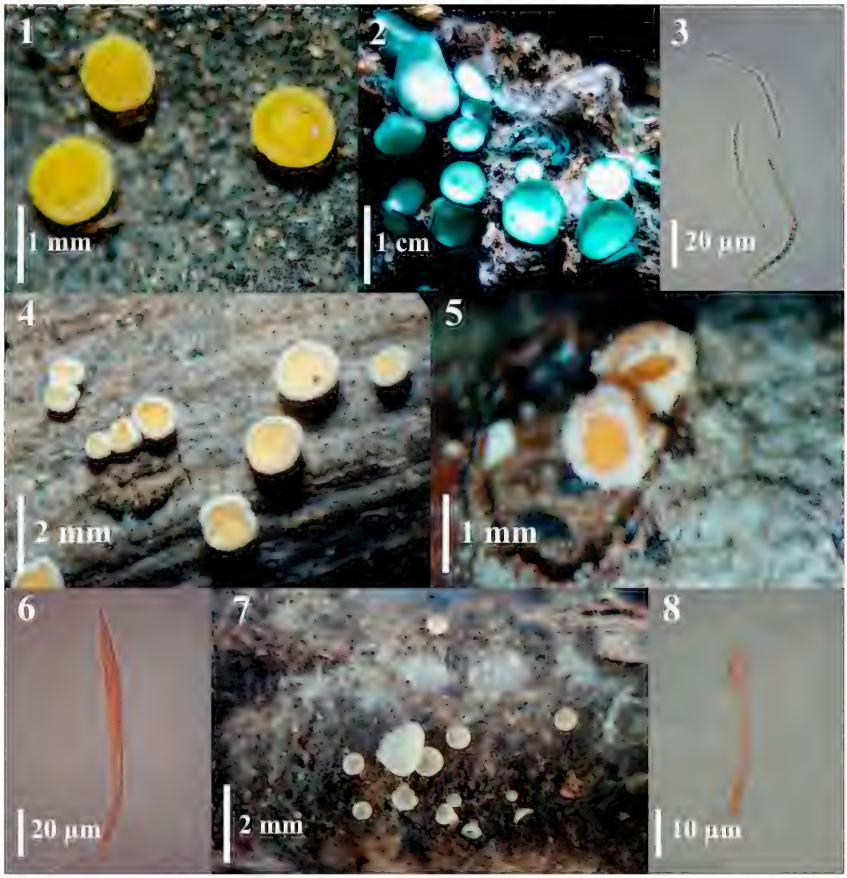
Lachnum brasiliense (Mont.) J.H. Haines & Dumont, Mycotaxon 19: 23 (1984)

Apothecia scattered on bark, light to dark yellow hymenium, 1.2 mm wide disc, shallow receptacle, shaped cup with a hairy short stalk, white to pale buff color and a blue-black base. Hairs cylindrical with obtusely rounded tips, (52–)60–75(–80) × 3–4 µm septate, thin walled, granulated. Asci cylindrical, (66–)78–82(–84) × 7–9 µm, 8-spored, hyaline, small blue pore in Melzer's reagent. Ascospores narrowly fusiform, cylindrical, usually slightly curved, occasionally becoming 3-septate, (30–)32–43 × 2.5–3 µm. Paraphyses cylindrical, (68–)72–91 × 2–3 µm, 1–3-septate.

HABITAT — Gregarious, lignicolous; in cloud forest at 1800 m.

MATERIAL EXAMINED — **GUATEMALA. CHIMALTENANGO DEPARTMENT**, San Andrés Itzapa Municipality, Aldea La Hierbabuena, 27 July 2007, Medel 1475 (XAL).

The dark to medium yellow ascomata clothed with white hairs and the blue-black base of the stalk are distinctive characters. *Lachnum brasiliense* is a common species in the tropics and has previously reported from Guatemala (Haines & Dumont, 1984) in pine forest. The material examined was found in cloud forest.



FIGURES 1–8. ***Bisporella citrina***: 1, ascospores. ***Chlorociboria aeruginosa***: 2, apothecia. ***Lachnum abnorme***: 3, ascospores; 4, apothecia. ***Lachnum cyphelloides***: 5, apothecia. ***Lachnum virgineum***: 6, paraphyses in Congo red. ***Hyalorbilia inflatula***: 7, apothecia. ***Orbilia juruensis***: 8, paraphyses. Bars: 1, 8 = 10 µm; 2 = 1 cm; 3, 6 = 20 µm; 4, 7 = 2 mm, 5 = 1 mm.

Lachnum cyphelloides (Pat.) J.H. Haines & Dumont, Mycotaxon 19: 30 (1984) FIG. 5

Apothecia yellow translucent, disc 1 mm wide × 1.2 mm of height, long stipitate, small white hairs to almost smooth. Hairs cylindrical, with obtusely rounded tips, 48–70 × 3–4 µm septate, hyaline, thin wall, granulated. Asci cylindrical 66–67 × 6–7 µm, small blue pore in Melzer's reagent. Ascospores fusiform, 31–40 × 1.5–2 µm hyaline. Paraphyses lanceolate, (68–) 72–91 × 2–3 µm hyaline, 1–3-septate.

HABITAT — Gregarious, lignicolous; in cloud forest at 1800 m.

Material examined — **GUATEMALA. Guatemala Department**, Universidad de San Carlos de Guatemala, University Campus, Parque Ecológico Las Ardillas, 27 July 2005, Medel 1465-A (XAL); 28 July 2005, Medel 1446 (XAL, MICG).

According to Haines & Dumont (1984), *L. cyphelloides* is covered with white hair, has a yellow pale disc, and its stipe lacks a blue-black base. The species is distributed in Central America, northern South America, and the Lesser Antilles (Haines & Dumont, 1984; Piepenbring 2006). This is first record for Guatemala.

Lachnum virgineum (Batsch) P. Karst., Bidr. Känn. Finl. Nat. Folk 19: 169 (1871)
Fig. 6

Apothecia concave smooth disc, 1–1.5 mm in diameter, white to creamy white, and margin enrolled when it dries. Central stipe, covered with white hairs, 1–1.2 mm of height. Hairs cylindrical, with obtusely rounded tips, 75–100 × 4–5 µm septate, thin walled, granulated hyaline. Asci cylindrical asci, 8-spored, 34–45 × 4–5µm, thin walled, small blue pore in Melzer's reagent. Ascospores fusiform, 6–8 × 1.5–2 µm, thin walled, hyaline. Paraphyses lanceolate, 70–100 × 5–6 µm, septate, hyaline, longer than the asci.

Habitat — Gregarious, lignicolous; in tropical forest at 160 m.

Material examined — **GUATEMALA. Alta Verapaz Department**, Cobán Municipality, Ecorregión Lachuá, 17 September 2005, López y Quezada 2473 (XAL).

Lachnum virgineum is characterized by apothecia covered by straight, white granulate hairs. Spooner (1987) reported the species from North and South America, Europe, North Africa, Central Asia, India, Japan, Papua New Guinea, and Australia. This is a new record for Guatemala.

Hypocreales

Ophiocordyceps gracilis (Grev.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, Stud. Mycol. 57:43 (2007)

Stromata with long cylindrical apices terminating in 5 × 3 mm heads, red ochraceous, with dark dotted perithecial ostioles. Stipe yellow-olive. Asci with many individual spores, 250–280 × 6 µm, pore not blue in Melzer's reagent. Ascospores cylindrical, smooth, 7–10 ×1–2 µm, hyaline, lined chainlike upon each other within the ascus. Paraphyses not observed.

Habitat — Solitary, on larvae of *Lepidoptera*; in cloud forest, 1800 m.

Material examined — **GUATEMALA. Chimaltenango Department**, San Andrés Itzapa Municipality, Aldea La Hierbabuena, 21 June 2007, Medel 1466 (XAL).

Our specimen was consistent with the descriptions by Mains (1958), Dennis (1978), and Breitenbach & Kränzlin (1984). *Ophiocordyceps gracilis* is always associated with lepidopteran larvae. This is the first report for Guatemala.

Orbiliales

Hyalorbilia inflatula (P. Karst.) Baral & G. Marson, Micologia 2000 (Trento): 44 (2000) FIG. 7

Apothecia less than 1.5 mm wide, orange to yellow color, with rolled margin. Asci cylindric-clavate, rounded apex, 8-spored, 21–23(–29) × 3–3.5(–4) µm, pore not blue in Melzer's reagent. Ascospores cylindrical, straight or slightly curved, rounded ends, 4–8 × 0.8–1 µm. Paraphyses cylindrical hyaline, 18–21 × 2(–2.5) µm, septate, with apices encrusted to form a thin epithecial layer.

HABITAT — Gregarious, lignicolous; in cloud forest and *Cupressus* forest at 1800 m.

MATERIAL EXAMINED — **GUATEMALA. CHIMALTENANGO DEPARTMENT**, San Andrés Itzapa Municipality, Aldea La Hierbabuena, 27 June 2007, Medel 1467, 1484 (XAL); **GUATEMALA DEPARTMENT**, Universidad de San Carlos de Guatemala, University Campus, Parque Ecológico Las Ardillas, 28 June 2007, Medel 1485-B (XAL, MICG).

Our specimen agreed with the description by Spooner (1987). Liu et al. (2006) note that *Hyalorbilia inflatula* is widely distributed in China. The species has also been recorded from Venezuela (Mardones-Hidalgo & Iturriaga 2011). This is the first report for Guatemala.

Orbilia juruensis Henn., Hedwigia 43: 270 (1904) FIG. 8

Apothecia superficial, sessile or subsessile, disc convex or undulate, with crenulate margin, translucent orange-yellow, smooth, 1 mm diameter. Receptacle smooth, concolorous or slightly paler than the disc, thin at the margin and thicker towards the centre. Asci cylindric-clavate , broadest at the apex and tapered downwards to a narrow base which is sometimes forked, apex truncate or obtusely rounded, 29–37 × 3.5–4 µm, pore not blue in Melzer's reagent. Ascospores straight or slightly inequilateral to fusoid, hyaline, (6–) 7–9(–10) × 1–1.5 µm, not septate. Paraphyses capitate, tips up to 3.5–4 µm diameter, 21–30 × 2–3 µm, 1–2-septa in the lower part.

HABITAT — Gregarious, lignicolous; in *Cupressus* forest at 1500 m.

MATERIAL EXAMINED — **GUATEMALA. GUATEMALA DEPARTMENT**, Universidad de San Carlos de Guatemala, University Campus, Parque Ecológico Las Ardillas, 28 June 2007, Medel 1491 (XAL).

This species is known form South America, Australasia, and Solomon Island (Spooner 1987), Argentina (Wright & Wright 2005), and Panama (Piepenbring 2006). This is the first record for Guatemala.

Pezizales

Gyromitra infula (Schaeff.) Quél., Enchir. Fung.: 272 (1886)

Ascoma bilobate or saddle-shaped, generally fused with the stipe. The hymenium covers the whole surface, which is cinnamon to dark brown. The

interior is hollow and whitish. The stipe is smooth, often somewhat furrowed or pitted, and finely felty towards the base. Asci cylindrical, (140–)150–250 × 15–17 µm. Ascospores elliptical, smooth, with 2 guttules, hyaline, 18–22 × 8–10 µm. Paraphyses clavate, 80–100 × 7–10 µm.

Habitat — Solitary or gregarious, humicolous; in *Pinus–Abies* forest, 2900 m.

Material examined — **GUATEMALA. Totonicapan Department**, Totonicapan Municipality, Mercado municipal de Totonicapan, 18 September 2001, Morales 295 (MICG); 19 August 2006, Morales 561 (MICG); Totonicapan Municipality, Aldea Panquix, 31 August 2003, Morales 485 (MICG).

The bilobate or saddle-shaped ascoma is characteristic of this species (Abbott & Currah 1997). Bran et al. (2003) previously reported this species from Guatemala, and it is also known from Mexico (Medel 2005).

Helvella acetabulum (L.) Quél., Enchir. Fung. 275 (1886)

Ascoma cup-shaped with an irregular margin, 25–50 mm broad; fertile surface dull brown. Lower surface smooth to subglabrous, light brown with conspicuous pallid to cream raised ribs extending from the stipe base to middle of the cup; thick whitish deeply ridged. Asci cylindrical, 200–300 × 14–17 µm. Ascospores broadly elliptical, hyaline, 15–21(–22) × 11–15 µm, smooth, with one guttule. Paraphyses with rounded tips, 5–6 µm thick.

Habitat — Solitary, humicolous; in pine–oak forest at 2300 m.

Material examined — **GUATEMALA. Chimaltenango Department**, Tecpan Municipality, Mercado municipal de Totonicapan, 7 October 1999, Morales 47 (MICG)

The ridged pale brown apothecium is characteristic of this species (Calonge & Arroyo 1990, Abbot & Currah 1997). The species is known from Canada (Abbot & Currah 1997), China (Zhuang 2004), Mexico (Medel & Calonge 2004; Vite-Garín et al. 2006) and Spain (Calonge & Arroyo 1990). This is the first record for Guatemala.

Xylariales

Annulohypoxylon thouarsianum (Lév.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, Mycologia 97: 861 (2005) Figs 9–10

Stromata sessile, globose, 12 mm wide × 7 mm high, with papillate ostioles in the middle of a deep ring, surface dark brown to black, carbonaceous, context fibrous woody, radially lined to faintly concentrically zoned grey and brown. Asci not observed. Ascospores inequilateral narrowly elliptical to slightly fusoid, light to medium brown, smooth, 23–27 × 6–7 µm, with a germ slit spore length on the flattened side. Paraphyses not seen.

Habitat — Gregarious, lignicolous; in oak forest.

Material examined — **GUATEMALA. Chimaltenango Department**, Tecpán Municipality, Astillero Municipal, 21 July 2007, Medel 1436 (XAL).

FIGURES 9–14. ***Annulohypoxylon thouarsianum***: 9, ascospores in KOH 5%; 10, stromata. ***Daldinia concentrica***: 11, ascospores in 5% KOH. ***Diatrypella pulvinata***: 12, ascospores in 5% KOH. ***Poronia pileiformis***: 13, ascus with blue pore + in Melzer's solution; 14, stromata (ostiole and perithecia). Bars: 9 = 20 µm; 10, 14 = 1 cm; 11 = 15 µm; 12 = 10 µm; 13 = 40 µm.

The species was first recorded from Guatemala by Sharp (1948) as *Hypoxylon malleolus* Berk. & Ravenel,, a synonym of *A. thouarsianum* (Ju & Rogers 1996, Hsieh et al. 2005). This is a common xylariaceous species with greenish pigments in KOH and papillate ostioles in the middle of a deep disc (Ju & Rogers 1996; Medel 2002). The species is also known from Mexico (Pérez-Silva 1987; Medel 2002) and Panama (Piepenbring 2006).

Daldinia concentrica (Bolton) Ces. & De Not., Comm. Soc. Crittog. Ital. 1: 197 (1863) FIG. 11

Stromata, globose circular to oval shaped 20–30 mm wide, sessile and, attached to the substrate, hard surface, reddish-brown to blackish young, and finally black. Asci cylindrical, 100–130 × 7–8 µm, uniseriate. Ascospores broadly elliptical to bean shape, dark brown, germ line straight, 13–15 × (6–) 6.5–7 µm. Paraphyses not seen.

HABITAT — Gregarious, lignicolous; in oak forest.

MATERIAL EXAMINED — **GUATEMALA. CHIMALTENANGO DEPARTMENT**, Tecpan Municipality, Astillero Municipal, 21 July 2007, Medel 1423 (XAL).

The important diagnostic characters of this species are the concentric lines in the stromata, and purple stromatal pigment in 5% KOH (Ju et al. 1997; Rogers et al. 1999). In Guatemala two species have been cited: *D. vernicosa* (Bran et al. 2003) and *D. fissa* (Morales et al. 2006). *Daldinia concentrica,* not previously reported for Guatemala, is also known from Mexico (Pérez-Silva 1978) and Europe, India, New Zealand, Taiwan, and the United States of America (Ju & Rogers 1997).

Diatrypella pulvinata Nitschke, Pyrenomyc. Germ. 1: 72 (1867) FIG. 12

Stromata pulvinate to oval, ostioles 3–5 sulcate, with endostromatic yellow whitish tissue. Asci multispored. Ascospores 7–9 × 1.5–2 µm, slightly curved (allantoid), hyaline, with 2 guttules. Paraphyses not seen.

HABITAT — Gregarious, lignicolous; in *Cupressus* forest at 1500 m.

MATERIAL EXAMINED — **GUATEMALA. GUATEMALA DEPARTMENT**, Universidad de San Carlos de Guatemala, University Campus, Parque Ecológico Las Ardillas, 28 July 2007, Medel 1489 (XAL).

The black sessile and pulvinate stromata with papillate ostioles are typical. *Diatrypella pulvinata* is known from Costa Rica, Europe, and the United States of America (Glawe & Rogers 1984; Chacón & Humaña 2006). This is the first record for Guatemala.

Poronia pileiformis (Berk.) Fr., Nova Acta R. Soc, Scient. Upsal., Ser. 3, 1: 129 (1851) FIGS 13–14

Stromata simple to branched, at the top of the stalk it has a small umbelliform 4–6 mm broad head, beige with dark dots (perithecial ostioles), smooth and leathery, later becoming hard and brittle. Ostioles papillate. Stipe dark brown to black, 60–70 mm high × 1–6 mm wide, smooth or ribbed with a thickened base. Asci cylindrical, 100–130 × 4–5 µm, pore blue in Melzer's reagent. Ascospores ellipsoid, dark brown, 7–11 × 4–5 µm, with a straight germ line as long as the length of the spore. Paraphyses not seen.

HABITAT — Gregarious, fimicolous, in tropical forest at 160 m.

MATERIAL EXAMINED — **GUATEMALA. ALTA VERAPAZ DEPARTMENT**, Cobán Municipality, Ecorregión Lachuá, 19 September 2005, López y Quezada 2473 (XAL).

This species is distinguished by the simple or branched stromata with a beige head with black papillate ostioles, growing on dung (Dennis 1957; San Martín et al. 1998). It is known from Costa Rica, Mexico, Peru, and Philippines. This is the first record for Guatemala

Discussion

With the new records, there are now 44 ascomycete species known from Guatemala (TABLE 1). The species encompass 26 genera of which *Helvella*

(5) and *Morchella* (4) provided the most records. The *Pezizales* was the most diverse order with 11 genera and 18 species. About 50% of the 200 specimens collected during the last survey in 2007 might represent new records or even new species.

The continued study of ascomycetes and other fungi from Guatemala will improve the knowledge of these organisms. This is essential because deforestation threatens to decrease the fungal biodiversity in this country. Recent deforestation data from Guatemala indicate a net 2006-2010 deforestation rate of over 38,000 ha / year (Regalado et al. 2012).

Acknowledgments

The authors thank to M.C. Bran (Universidad San Carlos de Guatemala) for supporting the expedition to collected the studied material of this study. Thanks to Dr. Gastón Guzmán and Juan Lara (INECOL) for providing literature and facilities to consult the herbarium, and to the reviewers Dra. Evangelina Pérez Silva (Universidad Nacional Autónoma de México) and Dra. Cecilia Carmarán (Universidad de Buenos Aires), who significantly improved this manuscript. Special thanks to Dr. Shaun Penycook for his critical review.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.87

Volume 124, pp. 87–99 *April–June 2013*

Coleosporium in Europe

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Abstract — The species of *Coleosporium* (*Uredinales*) occurring in Europe are not clearly differentiated morphologically. Most taxa in Europe have been combined into formae speciales (ff.spp.) of *Coleosporium tussilaginis* by the majority of researchers. This study includes the taxa *C. doronici*, *C. inulae*, and *C. telekiae* as further ff.spp. in *C. tussilaginis* and redefines the existing ff.spp. *campanulae-rapunculoidis* and *senecionis-silvatici*. It is hoped that this treatment will establish a pragmatic basis on which future taxonomic work can be built.

Key words — rust fungi, cryptic species, host specialism

Introduction

Coleosporium Lév. is a genus of *Uredinales* G. Winter (rust fungi) with the aecial stage parasitic on two-needle pines (*Pinus* spp.) and the telial stage on members of a wide range of angiosperm species. Its distribution is mostly limited to the northern hemisphere, where it is widespread and common in Europe, Asia, and North America (Farr & Rossman 2013, GBIF 2013). However, specimens have also been reported from South America (Arthur 1918, Hennen 2005) and New Zealand (McKenzie 1998). Their presence on two-needle pines has not been confirmed in these southern areas and specimens from Chile were reported to lack the teleomorph, possibly as a result (Arthur 1918). In New Zealand it is likely that already infected hosts from the northern hemisphere were planted or intercepted, and there is one reference of introduced *Coleosporium* rust on an indigenous species but no infections on two-needle pines have been reported (McKenzie 1998, Farr & Rossman 2013).

Léveillé established the genus in 1847. In a short note, he devised a system to bring more clarity into the confusing number of identical names of clearly distinct species and species with multiple names on account of their occurrence on a number of host plant species or genera (Léveillé 1847).

The type species is *Coleosporium campanulae* (lectotype, Laundon 1975).

Table 1. Distribution of *Coleosporium* in host plant families in Europe

Plant Family	Host genera	Host species	Predominant host genus
Asteraceae	35	110	*Senecio* (34 host taxa)
Campanulaceae	10	82	*Campanula* (63 host taxa)
Orobanchaceae	8	38	*Euphrasia* (16 host taxa)
Ranunculaceae	2	9	
Pinaceae	1	10	
Casual hosts & Misdeterminations			Notes
Tropaeolaceae	1	3	non-native plants
Orchidaceae	1	1	border intercept plant
Solanaceae	1	1	South American plants
[*Amaranthaceae*]	[1]	[1]	? misidentified host
[*Amarylidaceae*]	[1]	[1]	? cross-contamination from a common host
[*Boraginaceae*]	[2]	[2]	= *Melampsorella symphyti*
[*Caryophyllaceae*]	[1]	[1]	= *Caeoma coronariae*
[*Rosaceae*]	[1]	[1]	= *Pucciniastrum agrimoniae*

Most authors agree that the European taxa of *Coleosporium* are indistinguishable in their morphology (e.g. Sydow & Sydow 1915, Hylander et al. 1953, Gäumann 1959, Wilson & Henderson 1966, Boerema & Verhoeven 1972). Therefore, many authors used the host plant identity to distinguish species (Gäumann 1959, Minkevičius & Ignatavičiūtė 1991, Brandenburger 1994). Many current inventories treat all *Coleosporium* taxa distinguished in Europe as a single species and synonymise most of the original species into *Coleosporium tussilaginis* (Encyclopedia of Life 2012, Index Fungorum 2012). However, *C. doronici, C. inulae, C. ligulariae*, and *C. telekiae* have never been formally included in *C. tussilaginis*, although Hylander et al. (1953) did synonymise *C. ligulariae* with *C. senecionis*. The three remaining taxa are transferred to *C. tussilaginis* in the present study.

While some authors do not name the taxa included under *C. tussilaginis* (e.g. Majewski & Ruszkiewicz-Michalska 2008, Termorshuizen & Swertz 2011), others distinguish and name them (e.g., Gäumann 1959, Boerema & Verhoeven 1972, Encyclopedia of Life 2012, Bahcecioglu & Kabaktepe 2012), most often as formae speciales, i.e., biologically specialised forms (Eriksson 1894, Anikster 1984, Kirk et al 2008; see also the next paragraph below). The latter approach reflects the host specificity of *Coleosporium* analysed by extensive inoculation experiments (e.g., Klebahn 1904, 1924, Gäumann 1959, EURED names 2012). I follow this approach and extend it to all the European taxa.

Formae speciales (ff.spp.) are defined as taxa below the species level which are morphologically not or hardly distinguishable but are separated by host plant specificity. Nomenclature at this rank is not covered by the provisions of the International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012: Article 4 Note 4); therefore in this paper I present them as newly named taxa, rather than as "comb. nov."

This study covers the European taxa of *Coleosporium* on native plants. In the absence of molecular data, a morphological species concept is applied, extended by the use of discrete host plant ranges, in defining the sub-specific taxa of formae speciales, as described above. The aim is to present a pragmatic and effective basis for specimen sampling for molecular analysis, in order to generate data for further studies.

Materials & methods

The herbaria of Berlin (B), Paris (PC), Copenhagen (CP), St Petersburg (LE), Beltsville (BPI) and Edinburgh (E) were consulted in this research. Furthermore information was acquired from Zürich and Bern (Helfer et al. 2011) and from databases at Stockholm (Krypto-S 2012), BPI (Farr & Rossman 2013) and National Herbarium Netherlands (NHL 2012). Label information was extracted and compared with accepted host plant taxonomy using the Plant List (2012) database. Geographical information was checked using Fuzzy Gazetteer (Kohlschütter 2012) and Google Map (©2012 Google) and compiled according to the areas in the Atlas Florae Europaeae delimitations of Europe (Atlas Florae Europaeae 2012). Where reports were from non native or cultivated plants, this is indicated by the use of square brackets [ ]. Type material for *C. telekiae*, *C. doronici*, and *C. ligulariae* was obtained from Berlin (B).

Light microscopy: small portions of herbarium specimens were sectioned by hand and mounted in lactophenol-cotton blue for examination under a Carl Zeiss Axiophot© microscope with Carl Zeiss Axiocam© digital camera equipment. Measurements were achieved using the Axiocam software. Urediniospore dimensions as measured by light microscopy are reported in the following format: (min–) average +/-standard deviation (–max) length × breadth.

Scanning electron microscopy (SEM): small portions of herbarium specimens were removed and fastened to aluminium stubs with adhesive carbon tabs. Prior to sputter coating specimens were examined by a Carl Zeiss Stemi©2000 dissecting microscope, sorus sizes were measured and images taken with Carl Zeiss Axiocam© digital camera equipment as above. Specimens were coated with Palladium for 2min resulting in a deposition of approx 12nm and examined in a Carl Zeiss Supra©55VP SEM at 1-5kV and 5mm working distance. Measurements were achieved using the Axiocam software as above. Morphometric data were subjected to statistical analysis using the statistics package R (R Development Core Team 2011).

Details of the specimens examined are displayed with each taxon. Further information is available from the EURED specimens (2013) list.

Herbarium label images for B, PC, CP, LE and E used in this study can be viewed at the EURED website at E (Helfer et al. 2011). This website is continuously added to and improved.

Results

The herbarium data searches for European taxa of *Coleosporium* resulted in 249 host plant relationships (Table 1), represented in five families. A further seven taxa in five additional host families had been found on intercepted plants or as single occurrences and four taxa in three families were misinterpretations. In the past more than 50 valid names have been used for these rust taxa (EURED names 2012).

The microscopic examinations of the specimens were inconclusive with respect to urediniospore dimensions and sorus characters: while statistically significant differences could be found between most samples involving various host species there were also significant differences between collections of the same host/fungus relationship (EURED stats 2012). Furthermore, data ranges overlapped widely, indicating that urediniospore dimensions and sorus characters are unsuitable for identification purposes and for the characterisation or delimitation of taxa in this genus (EURED spores 2012). Similarly, spore surface characters observed by SEM revealed no distinctive features. It is therefore concluded that, morphologically, the taxa are all part of the same species.

Taxa present in Europe

Coleosporium tussilaginis (Pers.) Lév., in Orbigny, Dict. Univ. Hist. Nat. 12: 786 (1849) **f.sp. *tussilaginis*** Plate 1

≡ *Uredo tussilaginis* Pers., Syn. meth. fung. 1: 218 (1801).

= *Ustilago tussilaginis* Losa, Anales Jard. Bot. Madrid 5: 126 (1945).

Alternate hosts: *Pinus halepensis*, *P. mugo*, *P. nigra*, *P. pallasiana*, *P. pinaster*, *P. sylvestris* (Plate 1), [*P. echinata*, *P. ponderosa*, *P. rigida*].

Primary hosts: *Tussilago farfara*, [*Emilia coccinea*, *Erechtites glomeratus*], *E. hieraciifolius*, [*Euryops acraeus*, *E. evansii*, *Kleinia fulgens*, *K. grandiflora*].

European distribution: pan-European.

Specimens examined:
(as ***C. sp.***) PC0022213.
(as ***C. tussilaginis***) E00458054, E00458055, E00458056, E00458059, E00458060, E00458061.

Urediniospore size: (21.7–)28.3±3.2(–39.6) × (15.9–)20.8± 2.4(–26.6) µm.

Notes: Artificial infections were obtained on *Tropaeolum minus* (*Tropaeolaceae*) and *Schizanthus grahamii* (*Solanaceae*) (Gäumann 1959); weak infections were also obtained on plants of *Senecio vulgaris* (Klebahn 1924), calling into question the biological separation of this taxon from f.sp. *senecionis* discussed below. New specimens in many herbaria are deposited as *C. tussilaginis*, irrespective of host plant identity.

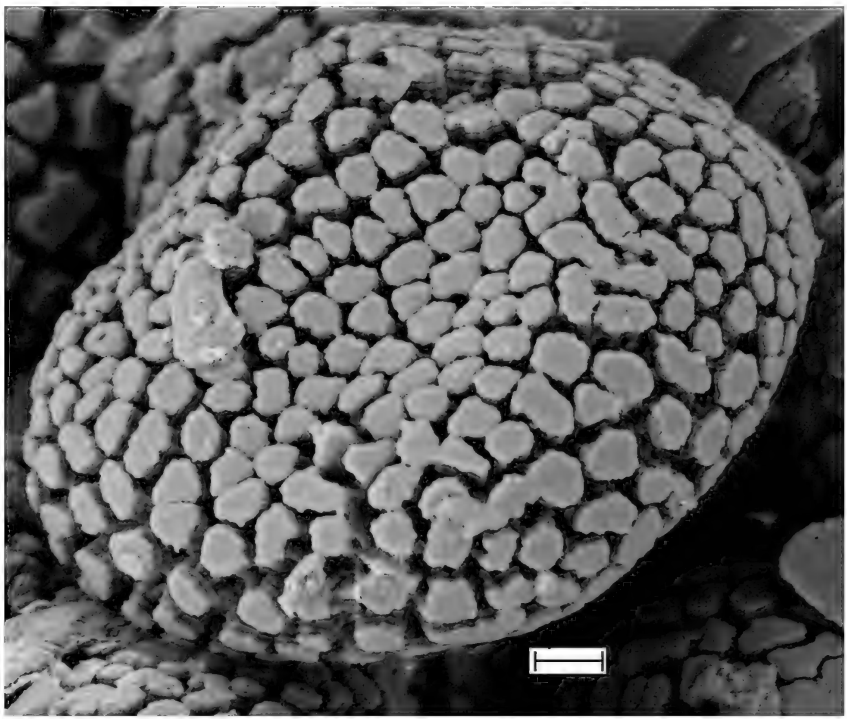

PLATE 1 Aeciospore of *Coleosporium tussilaginis* on *Pinus sylvestris*. SEM; bar = 2 µm.

Coleosporium tussilaginis* f.sp. *campanulae-rapunculoidis Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 7 (1972).

= *Coleosporium campanulae* (Pers.) Fr., Summa Vegetabilium Scandinaviae: 512 (1849), as "*campanulacearum*".

= *Coleosporium campanulae-rapunculoidis* Kleb., Wirtswechselnde Rostpilze: 365 (1904).

= *Coleosporium campanulae-rotundifoliae* Kleb., Wirtswechselnde Rostpilze: 366 (1904).

= *Coleosporium campanulae-trachelii* Kleb., Wirtswechselnde Rostpilze: 366 (1904).

= *Coleosporium tussilaginis* f.sp. *campanulae-rotundifoliae* Boerema & Verh., Neth. Jl Pl. Path. 78(Suppl. 1): 7 (1972).

= *Coleosporium tussilaginis* f.sp. *campanulae-trachelii* Boerema & Verh., Neth. Jl Pl. Path. 78(Suppl. 1): 7 (1972).

ALTERNATE HOSTS: *Pinus nigra*, *P. sylvestris*.

PRIMARY HOSTS: *Adenophora liliifolia*, [*A. triphylla*], *Asyneuma giganteum*, *A. limonifolium*, *Campanula alliariifolia*, *C. alpina*, [*C. americana*], *C. barbata*, *C. baumgartenii*, *C. bononiensis*, [*C. californica*], *C. carnica*, *C. carpatica*, *C. caucasica*, *C. cervicaria*, *C. cespitosa*, *C. cochleariifolia*, *C. drabifolia*, *C. erinus*, *C. glomerata*, [*C. grandis*], *C. grossekii*, *C. hagielia*, *C. hawkinsiana*, *C. hispanica*, *C. incurva*, *C. isophylla*, *C. lactiflora*, *C. lanata*, *C. latifolia*, *C. lusitanica*, *C. lyrata*, *C. macrorhiza* subsp. *gypsicola*,

C. medium, *C. moesiaca*, *C. patula*, *C. pelia*, *C. persicifolia*, *C. persicifolia* subsp. *subpyrenaica*, *C. pulla*, [*C. punctata*], *C. pyramidalis*, *C. rapunculoides*, *C. rapunculus*, *C. rhomboidalis*, *C. romanica*, *C. rotundifolia*, *C. rumeliana*, *C. rupestris*, *C. sarmatica*, *C. saxatilis*, *C. scheuchzeri*, *C. serrata*, *C. sibirica*, *C. sparsa*, *C. sparsa* subsp. *sphaerothrix*, *C. spathulata*, *C. speciosa* subsp. *affinis*, *C. speciosa*, *C. stenosiphon*, *C. stevenii*, *C. tatrae*, *C. tomentosa*, *C. trachelium*, *C. transsilvanica*, *C. versicolor*, *C. wanneri*, *Jasione montana*, *Legousia hybrida*, *L. speculum-veneris*, [*Lobelia cardinalis*, *L. xalapensis*], *Michauxia campanuloides*, *M. laevigata*, *Petromarula pinnata*, *Phyteuma betonicifolium*, *P. nigrum*, *P. orbiculare*, *P. scheuchzeri*, *P. spicatum*, [*Triodanis perfoliata*, *Wahlenbergia denticulata*].

European distribution: Au, Be, Bel, Br, Bu, Cg, Cs, Ct, Da, EU, Fe, Ga, Ge, Gr, He, Ho, Hs, Hu, Is, It, La, Lu, Mo, No, Po, Rm, Rus, Se, Sk, Su, Tu, Uk.

Specimens examined:
(as ***C. campanulae***) E00159053, E00458064, PC0022266, PC0022293, PC0022305, PC0022308, PC0022317, PC0022324, PC0022354.

Urediniospore size: (12.0–)20.5±4.8(–30.2) × (9.0–)14.9±3.2(–26.4) µm.

Notes: Boerema & Verhoeven (1972) treat this in three separate formae speciales: f.sp. *campanulae-rapunculoidis*, f.sp. *campanulae-rotundifoliae,* and f.sp. *campanulae-trachelii*. Gäumann (1959) lists several overlapping host ranges and infections on unrelated angiosperms, such as *Tropaeolum minus*, and I see no justification to maintain these three special forms.

Coleosporium tussilaginis f.sp. *doronici* S. Helfer, f.sp. nov.

MycoBank MB804294

= *Coleosporium doronici* Namysl., Spraw. Kom. fizyogr. Akad. Umijetn. Krakowie 45: 125 (1911).

Alternate hosts: *Pinus* sp. (presumed).

Primary hosts: *Doronicum austriacum*, *D. columnae*.

European distribution: Au, Cs, Hu, Po, Rm.

Specimens examined:
(as ***C. doronici***) B700015048, B700015049, B700015050, B700015051, B700015052, B700015053, PC0022411, PC0022412.

Urediniospore size: (10.8–)27.8±3.8(–34.1) × (7.0–)19.1±3.6(–24.8) µm.

Notes: This appears to be a geographically as well as biologically separate special form on *Doronicum* sp. (Müller 2003), since its hosts are widely distributed over Europe (Atlas Florae Europaeae 2012). The report in Gäumann (1959) that this rust occurs in Spain is based on a misunderstanding of the geographical name Galicia ("Galizien"), in fact referring to a region in the Tatra mountain border region of Poland.

Coleosporium tussilaginis f.sp. *inulae* S. Helfer, f.sp. nov.

MycoBank MB804295

= *Uredo erigerontis* Req. ex Duby, Bot. Gall. Edn 2: 893 (1830), as "*erigeronis*".

= *Coleosporium inulae* Rabenh., Bot. Zeitung 9: 455 (1851).

= *Coleosporium carpesii* Sacc., Rivista Period. Lav. Regia Accad. Sci. Lett. Arti Padova 24: 208 (1874).

= *Coleosporium jasoniae* Gonz. Frag., Trab. Mus. Nac. Cienc. Nat. Madrid, é Bot. 9: 23 (1916).

= *Coleosporium asterisci-aquatici* (Sacc.) Syd. & P. Syd., Ann. mycol. 19: 249 (1921)

ALTERNATE HOSTS: *Pinus halepensis*, *P. sylvestris.*

PRIMARY HOSTS: *Asteriscus aquaticus*, *Carpesium cernuum*, *Centaurea hierapolitana*, *Dittrichia graveolens*, *D. viscosa*, *Inula aschersoniana*, *I. aspera*, *I. britannica*, *I. candida*, *I. conyza*, *I. ensifolia*, *I. germanica*, *I. helenioides*, *I. helenium*, *I. helvetica*, *I. heterolepis*, *I. hirta*, *I. methanaea*, *I. oxylepis*, *I. parnassica*, [*I. royleana*], *I. salicina*, *I. spiraeifolia*, *Jasonia tuberosa.*

EUROPEAN DISTRIBUTION: Al, Au, Bl, Bu, Cg, Co, Cs, Ct, Da, Es, Fe, Ga, Ge, Gr, He, Hs, Hu, It, La, Lu, Mk, No, Po, Rm, Rus, Su, Tu, Uk.

SPECIMENS EXAMINED:
(as ***C. inulae***) E00458051, E00458052, E00458053, E00458063.
(as ***Uredo erigeronis***) PC0022413, PC0022415.

UREDINIOSPORE SIZE: (21.2–)30.3±3.3(–39.5) × (15.7–)23.4±3.1(–30.7) µm.

NOTES: A special form on most of the genera of tribus *Inuleae* Cass. of the *Compositae*. Significant damage caused by this rust on elecampane (*Inula helenium*) grown for medicinal use has been reported in Serbia (Pavlovic et al. 2003).

Coleosporium tussilaginis* f.sp. *melampyri Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 8 (1972).

= *Coleosporium melampyri* (Rebent.) Kleb., Annls Sci. Nat., Bot., sér. 4, 4: 136 (1854).

ALTERNATE HOSTS: *Pinus mugo, P. sylvestris.*

PRIMARY HOSTS: *Melampyrum arvense*, *M. nemorosum*, *M. polonicum* Soó, *M. pratense*, [*Schizanthus* sp.].

EUROPEAN DISTRIBUTION: Au, Bel, Br, Cs, Da, Fe, Ga, Ge, He, Ho, Hs, Hu, It, No, Po, Rm, Rus, Sl, Su.

SPECIMENS EXAMINED:
(as ***C. euphrasiae***) PC0022164.
(as ***C. melampyri***) E00458065, E00458066, E00458067, E00458068.
(as ***C. tussilaginis***) E00458050.
(as ***C. rhinanthacearum***) PC0022434.

UREDINIOSPORE SIZE: (20.6–)25.9±2.6(–32.4) × (13.3–)18.9±2.2(–24.2) µm.

NOTES: Heavy infections of *Melampyrum* have been reported in Finland (Pohjakallio & Vaartaja 1948). This rust was found to infect cultivated *Schizanthus* sp. (*Solanaceae*) in Sweden (Hylander et al. 1953) and Norway (Gjaerum 1974); please also see notes to f.sp. *rhinanthacearum* below.

Coleosporium tussilaginis* f.sp. *petasitis Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 8 (1972).

= *Coleosporium petasitidis* Lév., Annls Sci. Nat., Bot., sér. 3, 8: 373 (1847).

= *Coleosporium petasitis* de Bary, Microscopic fungi: 213 (1865).

ALTERNATE HOSTS: *Pinus mugo*, *P. nigra*, *P. sylvestris.*

Primary hosts: *Petasites albus*, *P. frigidus*, *P. frigidus* var. *palmatus*, *P. hybridus*, [*P. japonicus*, a naturalized alien in central and northern Europe], *P. kablikianus*, *P. paradoxus*, *P. radiatus*, *P. spurius*.

European distribution: Au, Br, Bu, Cs, Da, Fe, Ga, Ge, Gr, Hb, He, Ho, It, La, No, Po, Rm, Rus, Sk, Su.

Specimens examined:
(as ***C. petasitis***) E00458069, E00458070, E00458071.

Urediniospore sizes: (18.4–)26±2.3(–31.6) × (14.3–)20.7±2.2 (–26.6) µm.

Notes: Together with f.sp. *senecionis*, f.sp. *tussilaginis*, and f.sp. *doronici*, this rust develops on members of the tribus *Senecioneae* Cass. of the *Compositae*.

Coleosporium tussilaginis* f.sp. *pulsatillae Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 8 (1972).

= *Uredo tremellosa* var. *pulsatillae* F. Strauss, Ann. Wetterauischen Ges. Gesammte Naturk. 2: 89 (1811) ["1810"].

= *Coleosporium pulsatillae* (F. Strauss) Fr., Summa Vegetabilium Scandinaviae: 512 (1849), as "*pulsatillarum*".

Alternate hosts: *Pinus sylvestris*.

Primary hosts: *Anemone alpina*, *A. halleri*, *A. montana*, [*A. patens*], *A. pratensis*, *A. pulsatilla*, *A. slavica*, *Pulsatilla grandis*, *P. nigricans*.

European distribution: Au, Cs, Da, Fe, Ga, Ge, He, Ho, Hu, It, La, No, Po, Rm, Rus, Sk, Su, Uk.

Specimens examined:
(as ***C. pulsatillae***) E00458057, E00458062.

Urediniospore size: (20.8–)28.7±4.2(–40.3) × (15.5–)19.6±2.2(–24.4) µm.

Notes: This special form occurs on members of the *Ranunculaceae*. Despite its morphological likeness, many authors (Braun 1981, 1982, Su et al. 2012) consider this as a separate species. I concur with Boerema & Verhoeven (1972) in the treatment as a special form, as no reliable distinction can be made using morphometric methods.

Coleosporium tussilaginis* f.sp. *rhinanthacearum Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 8 (1972).

= *Coleosporium rhinanthacearum* (DC) Lév., Annls Sci. Nat., Bot., sér. 3 8: 373 (1847).

= *Coleosporium flavum* Bonord., Abh. Naturf. Ges. Halle: 186 (1860).

= *Coleosporium euphrasiae* (Schumach.) G. Winter, Rabenh. Krypt.-Fl., Edn 2, 1(1): 246 (1881) ["1884"].

Alternate hosts: *Pinus mugo*, *P. sylvestris*.

Primary hosts: *Bartsia alpina*, [*B. lutea*, *B. trixago*], *B. viscosa*, *Euphrasia* × *reuteri*, *E. arctica*, *E. brevipila*, *E. hirtella*, *E. liburnica*, *E. micrantha*, *E. minima*, *E. nemorosa*, *E. parviflora*, *E. pectinata*, *E. picta* subsp. *kerneri*, *E. rostkoviana*, *E. rostkoviana* var. *fennica*, *E. salisburgensis*, [*E. striata*], *E. tricuspidata*, *Odontites litoralis*, *O. purpureus*, *O. vulgaris*, *Orthantha lutea*, *Parentucellia viscosa*, *Pedicularis palustris*, *Rhinanthus*

aestivalis, *R. alectorolophus*, [*R. apterus*], *R. glaber*, *R. glacialis*, *R. pulcher* subsp. *alpinus*, *R. riphaeus*, *R. serotinus*.

European distribution: Au, Be, Bel, Br, Bu, Cg, Cs, Da, Fe, Ga, Ge, Hb, He, Ho, Hs, Hu, It, La, Lu, No, Po, Rm, Rus, Sk, Su, Uk.

Specimens examined:
(as ***C. euphrasiae***) PC0022172, PC0022176, PC0022178, PC0022200, PC0022420.
(as ***C. flavum***) PC0022202.
(as ***C. rhinanthacearum***) E00458082, PC0022434, PC0022459.

Urediniospore size: (19.2–)22.9±2.0(–28.4) × (14.1–)17.9±1.7(–22) µm.

Notes – Besides f.sp. *melampyri* this is the second forma specialis on these more advanced members of the *Orobanchaceae* (Tank et al. 2006). In many herbaria the specimens of rusts on *Rhinanthus*, *Euphrasia*, *Melampyrum* and related hosts are labelled or arranged under any of the names *Coleosporium euphrasiae, C. melampyri, C. rhinanthacearum, C. tussilaginis*.

Coleosporium tussilaginis* f.sp. *senecionis-silvatici Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 9 (1972)

= *Coleosporium senecionis* f.sp. *senecionis-silvatici* Wagner ex Gäum., in Beitr. KryptogFlora Schweiz 12: 124. 1959.
= *Coleosporium senecionis* (Pers.) Fr., Summa Vegetabilium Scandinaviae: 512 (1849).
= *Coleosporium cacaliae* G.H. Otth, Mitt. naturf. Ges. Bern: 179 (1865).
= *Coleosporium ligulariae* Thüm., Bull. Soc. Imp. Nat. Moscou 52: 140 (1877).

Alternate hosts: *Pinus halepensis*, *P. mugo*, *P. nigra, P. pinaster*, *P. pinea*, *P. sylvestris*.

Primary hosts: *Adenostyles alliariae*, *A. alpina*, [*Delairea odorata*, *Farfugium japonicum* var. *giganteum*, *Hasteola suaveolens*], *Jacobaea alpina*, *J. arnautorum*, *J. erucifolia* subsp. *praealta*, *J. paludosa*, [*Ligularia fischeri*, *L. macrophylla*], *L. sibirica*, *Pallenis maritima*, *Parasenecio hastatus*, *Pericallis cruenta*, [*P. hybrida*, *Senecio candicans*], *S. carpetanus*, [*S. congestus*], *S. doria*, *S. doronicum*, *S. duriaei*, [*S. elegans*], *S. gallicus*, [*S. glaberrimus*], *S. hercynicus*, [*S. inaequidens*], *S. incrassatus*, *S. jacobaea*, *S. leucanthemifolius*, *S. leucanthemifolius* subsp. *vernalis*, [*S. leucostachys*], *S. lividus*, *S. macrophyllus*, [*S. moorei*], *S. nemorensis*, *S. ovatus*, [*S. pulcher*], *S. pyrenaicus*, [*S. scandens*, *S. smithii*], *S. squalidus*, *S. subalpinus*, *S. sylvaticus*, [*S. tristis*], *S. umbrosus*, [*S. viravira*, *S. viscidulus*], *S. viscosus*, *S. vulgaris*, *Tephroseris palustris*.

European distribution: Au, Az, Be, Bl, Br, Bu, Cg, Co, Cs, Da, Fe, Ga, Ge, Gr, Hb, He, Ho, Hs, Hu, It, La, Lu, No, Po, Rm, Rus, Sk, Su, Uk.

Specimens examined:
(as ***C. cacaliae***) PC0022228.
(as ***C. ligulariae***) B700015041, B700015042, B700015044, B700015045, B700015046, B700015047.
(as ***C. senecionis***) E00458072, E00458073, E00458074, E00458075, E00458076, E00458077.

Urediniospore size: (18.6–)25.7±3.2(–37.1) × (11.8–)18.7±2.2(–26.4) µm.

Notes: This is probably a pan-European form. Its distinctness from f.sp. *tussilaginis* needs to be confirmed by detailed host range studies or molecular investigations (see notes there).

Coleosporium tussilaginis* f.sp. *sonchi Boerema & Verh., Netherlands J. Pl. Pathol. 78(Suppl. 1): 9 (1972).

= *Uredo tremellosa* var. *sonchi* F. Strauss, Ann. Wetterauischen Ges. Gesammte Naturk 2: 90 (1811) ["1810"]; see Hylander et al. (1953).

= *Coleosporium sonchi* Lév., Annls Sci. Nat., Bot., sér. 4 2: 190 (1854).

= *Coleosporium sonchi-arvensis* (Pers.) Lév., in Orbigny, Dict. Univ. Hist. Nat. 12: 786 (1849).

Alternate hosts: *Pinus sylvestris.*

Primary hosts: *Aposeris foetida*, *Arnoglossum atriplicifolium*, *Crepis palaestina*, *C. tectorum*, *Dendroseris litoralis*, *Lactuca muralis*, *Lapsana communis*, *Picris cupuligera*, *Sonchus arvensis*, *S. asperl*, *S. brachyotus*, *S. oleraceus*, *S. palustris*, *S. tenerrimus*, *S. uliginosus*.

European distribution: Au, Bel, BH, Br, Bu, Cg, Cs, Da, Fe, Ga, Ge, Hb, He, Ho, Hs, It, La, Lu, No, Po, Rm, Rus, Sk, Su, Uk.

Specimens examined:
(as ***C. sonchi***) E00458078, E00458079.
(as ***C. sonchi-arvensis***) E00458080.

Urediniospore size: (15.9–)21.8±2.5(–29.6) × (9.6–)17.2±3.0(–23.9) µm.

Notes: This forma specialis is restricted to the tribus *Cichorieae* Lam. & DC. of the *Compositae*.

Coleosporium tussilaginis* f.sp. *telekiae S. Helfer, **f.sp. nov.**

MycoBank MB804296

= *Coleosporium telekiae* Thüm., Fungi austriaci exsiccati no. 850 (1873)

Alternate hosts: *Pinus* sp. (presumed)

Primary hosts: *Telekia speciosa*, *Xerolekia speciosissima*.

European distribution: Au, Bu, Cs, [Fe,] Ge, It, [No,] Po, Rm, Sk, Uk.

Specimens examined:
(as ***C. telekiae***) B700004933, B700010610, B700010611, B700015019, B700015020, B700015021, B700015022, B700015023, B700015024, B700015026, B700015027, B700015030, B700015033, B700015034, B700015035, B700015037, B700015038, B700015039, B700015040, E00458058.

Urediniospore size: (15.8–)22.6±3.0(–31.8) × (10.6–)17.2±2.4(–24.1) µm.

Notes: As well as in its natural range in Eastern Central Europe (Kokeš 2004, Dietrich 2009), this rust was recently found on cultivated *Telekia speciosa* in Western Norway (Gjaerum et al. 2008).

Discussion

The present study used the host plant taxonomy of the Plant List (2012) database and revised the host plant spread to at least 239 taxa in 55 genera represented in 4 angiosperm families as well as ten *Pinus* species as alternate hosts (Table 1). The most common host family is the *Compositae,* represented by 96 genera and 706 taxa worldwide and 35 genera and 110 taxa in Europe (Farr & Rossman 2013). Kenneth & Palti (1984), who published a detailed study of

the fungus-plant interactions of this family, found that globally *Coleosporium* was most widely distributed on the tribe *Heliantheae* with 14 genera affected followed by *Senecioneae* with 11 genera. Members of *Heliantheae* are not native in Europe, leaving *Senecioneae* as the most important host tribe, as is confirmed in this study.

To date few molecular investigations into *Coleosporium* have been published. A total of 32 sequences is currently deposited in GenBank (GeneBank 2013), mostly of the Asian/North American species *C. asterum* (Dietel) Syd. & P. Syd. Maier et al. (2003) compared four accessions of *Coleosporium* using the nuclear large subunit ribosomal DNA. In their neighbor joining analysis *C. asterum* (synonym *Stichopsora asterum* Dietel) was clearly separated from *C. cacaliae*, *C. campanulae*, and *C. tussilaginis*, which were not resolved.

Recent molecular studies have revealed the presence of cryptic species among morphologically indistinguishable specimens of *Melampsora* Castagne (Bennett et al. 2011, Milne et al. 2012), a rust genus of comparable host diversity, and it is probable that similar speciation is taking place in *Coleosporium*.

Conclusions

The genus *Coleosporium* is well defined within the *Uredinales*. Its taxa, however, are seriously confused. While host specificity appears to restrict the host range in some taxa, it does not seem to do so in others. Molecular taxonomic investigation of *Coleosporium* taxa is only in its infancy, and new initiatives are urgently needed to deal with the relationships in this genus.

Acknowlegements

The author acknowledges the support of the herbaria in B, CP, LE, PC, Z+ZT, BERN for access to specimens. The study was financially supported by SYNTHESYS (for visits to B [DE-TAF-924] and PC [FR-TAF-5178]) as well as a travel grant from the British Society for Plant Pathology to visit CP. Thanks are also expressed to Dr Reinhard Berndt and Prof. Salvatore Moricca for helpful comments during the peer review process and to the Nomenclature Editor.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.101

Volume 124, pp. 101–109 *April–June 2013*

Septoglomus titan, a new fungus in the *Glomeraceae* (*Glomeromycetes*) from Bahia, Brazil

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Abstract — A new fungus of the *Glomeraceae* found in the rhizosphere of *Agave sisalana* is described under the epithet *Septoglomus titan*. It forms large glomerospores, (243–)265 × 325(–400) µm in diameter. They generally are subglobose and have three spore wall layers: a semi-persistent short lived sub-hyaline to yellow-brown outer layer (0.5–1.4 µm thick), an adherent smooth light-yellow to orange-brown middle layer (2.5–5.1 µm), and a laminate smooth thick orange brown to dark red brown innermost layer (12.8–19.2 µm). Spore size and colors of the spore wall layers separate this species from other yellow-brown to dark-brown species.

Key words — *Glomerales*, *Agavaceae*, *Glomus* group A, crop system, rDNA

Introduction

Approximately 230 species of arbuscular mycorrhizal fungi (AMF; *Glomeromycota*) have been described, of which 118 exhibit glomoid spore development (sensu lato; Oehl et al. 2011a, Estrada et al. 2011). Historically, identification of the glomoid species was considered extremely difficult because of the limited morphological characters (Morton 1988), and the molecular approach has been used to solve the hypothetical separation into different genera, families, and orders (Walker & Schüßler 2004, 2007, Schüßler & Walker 2010). For this reason recent taxa from different glomeromycotan groups have been poorly described morphologically (Walker & Schüßler 2004, Schüßler & Walker 2010), including groups with diversified morphological data sets such as *Acaulospora, Kuklospora,* and *Ambispora* (Kaonongbua et al. 2010, Krüger et

al. 2011, Schüßler & Walker 2010). Recently Oehl et al. (2011a,b,c) combined significant morphological characters with concomitant molecular data to reorganize the *Glomeromycota*, especially the groups with glomoid spore development. This newly available morphological data set is being successfully used to identify distinct groups with glomoid spores (Estrada et al. 2011, Furrazola et al. 2011, Goto et al. 2012).

Glomoid species are very commonly found in Brazilian agrosystems (Silva et al. 2007, Goto et al. 2010, Maia et al. 2010, Nobre et al. 2010) with reports of many undescribed species (Carrenho et al. 2010) but with new descriptions rarely available. Studies on AMF diversity in *Agave sisalana* (common name: sisal) crop systems in Bahia State, Brazil, have revealed an undescribed species forming large spores with glomoid development, which we describe here as *Septoglomus titan*.

Material & methods

Study areas, soils sampling and soil parameters

Study areas were in the municipalities of São Domingos located at 12°48′S 41°37′W, and Ourolândia located at 10°58′S 41°05′W, both in the semi-arid region of Bahia State, in the Northeast Brazil. This semi-arid region is characterized by tropical dry climate (type Am of Köppen-Geiger; Kottek et al. 2006), with mean annual temperature of 30°C and precipitation of 400–600 mm. The vegetation was agriculturally managed sisal, a perennial culture of 40-year-old systems, in a Caatinga biome characterized by low tree stratum without a continuous canopy, trees and shrubs with thin stems and small or composite leaves, deciduous in the dry season (Queiroz 2006). The predominant local geology is characterized by a granite-greenstone formed by the Santa Luz complex, Archaean basement, and comprises an assemblage of migmatitic gneisses with subordinate granitoids. The fungus was found in soil samples collected around *Agave sisalana* roots in farms of both municipalities. Soil characteristics in Ourolândia were: pH ($CaCl_2$) = 6.4, organic matter = 13 g dm^3, available P = 18 mg dm^3 and in São Domingos were: pH ($CaCl_2$) = 6.3, organic matter = 32 g dm^3, and available P = 58 mg dm^3.

AMF bait cultures

The native AMF communities were cultured for two cycles (5 months each) with *Sorghum bicolor*, *Brachiaria decumbens,* and *Panicum miliaceum* in 500 mL pots filled with autoclaved sand substrate (250 g per pot) mixed with the natural field soil as AMF inoculum (250 g per pot), at the greenhouse of the Center for Agricultural, Environmental and Biological Research, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Brazil. Additionally, multiple glomerospores from the new species were separated and used as infective propagules in single species cultures on *S. bicolor*. The new fungus could successfully be propagated in bait cultures together with *Acaulospora scrobiculata*, *Ambispora appendicula, Claroideoglomus etunicatum, Entrophospora infrequens, Funneliformis mosseae, Glomus intraradices, G. sinuosum, Paraglomus brasilianum,* and *P. occultum*. However, no single species cultures were obtained from the fungus. Glomerospores isolated exclusively from the trap cultures were used for morphological and molecular analyses.

Morphological analyses

Spores of the new species were extracted as described in Sieverding (1991). Trap cultures were established according to Goto et al. (2012) modified by using Hoagland's solution without phosphorus. The spores were thereafter mounted in polyvinyl alcohol–lacto–glycerin (PVLG), in PVLG + Melzer's reagent, and in water (Brundrett et al. 1994, Spain 1990). The terminology used is based on recent papers (Furrazola et al. 2011, Goto & Maia 2006, Goto et al. 2012, Estrada et al. 2011)

Molecular analyses

DNA EXTRACTION: DNA was extracted from three single spores of the new species. Individual spores on a slide with a drop (5–10 µl) of ultrapure water were crushed with a needle. The resulting material was used directly in the PCR reactions.

AMPLIFICATION AND SEQUENCING: DNA extract was used as template for a semi-nested PCR using the primers ITS3 (White et al. 1990) – 28G2 (Silva et al. 2006) and LR1 (van Tuinen et al. 1998) – 28G2 consecutively. PCR reactions were carried out in a volume of 50 µl, containing 75 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1 µM of each primer, and 2 units of Taq™ DNA polymerase (Fermentas); cycling parameters were 5 min at 95°C (1 cycle), 45 s at 94°C, 1 min at 55°C, 1 min at 72°C (40 cycles), and a final elongation of 7 min at 72°C following the last cycle. The amplified products were purified with a PureLink PCR Purification Kit (Invitrogen), following the manufacturer's instruction and sequenced. Sequencing was provided by the Human Genome Research Center (São Paulo, Brazil).

SEQUENCE ALIGNMENT: Querying the National Center for Biotechnology Information databases with the BLASTn program, we verified that the sequences obtained from *S. titan* were affiliated with the *Glomerales* (*Glomeromycota*) before phylogenetic analysis. The AM fungal sequences (partial LSU rRNA) obtained in our laboratory were aligned with other glomeromycotean sequences from the GenBank using the program ClustalX (Larkin et al. 2007) and edited with the BioEdit program (Hall 1999) to obtain a final alignment. The sequences were deposited at GenBank under the accession numbers JQ312667–312669.

PHYLOGENETIC ANALYSES: Maximum parsimony (MP) analysis with 1000 bootstrap replications was performed using the Phylogenetic Analysis Using Parsimony (PAUP) program version 4 (Swofford 2003). Bayesian (two runs over 1×10^6 generations with a burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The model of nucleotide substitution (GTR + G) was estimated using Topali 2.5 (Milne et al. 2004). Sequences from *Claroideoglomus claroideum* and *C. etunicatum* were used as outgroup.

Results

Molecular analyses

Phylogenetic analyses of LSU rRNA gene sequences (FIG. 1) revealed that the new fungus is a *Septoglomus* species (former *Glomus* group Aa3 sensu Oehl et al. 2011a).

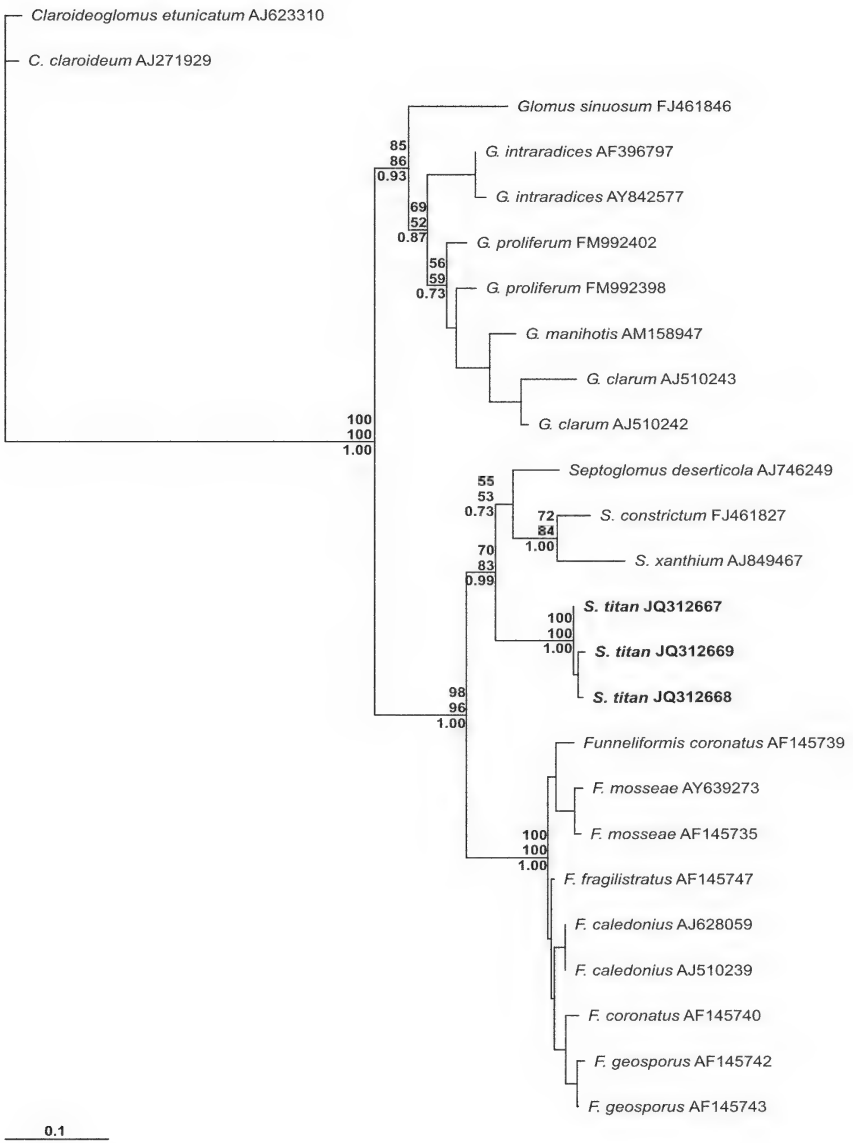
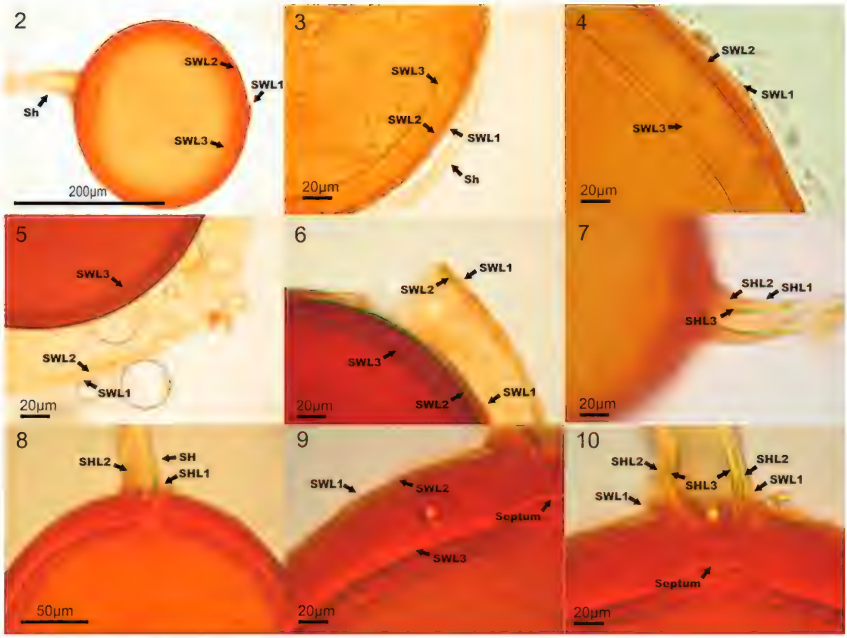


FIG. 1. Phylogenetic tree of the *Glomeraceae* based on LSU rDNA analysis and rooted by *Claroideoglomus claroideum* and *C. etunicatum*. Sequences are labeled with database accession numbers. Support values are from maximum parsimony (MP), maximum likelihood (ML) and bayesian analyses, respectively. *Septoglomus titan* sequences are in bold. Only topologies with ≥50% bootstrap values are shown. (Consistency Index = 0.62; Retention Index = 0.79).



FIGS 2–10. *Septoglomus titan*. **2.** Spore formed on subtending hypha (sh) with three spore wall layers (SWL1, SWL2, SWL3) continuous with subtending hyphal wall layers. **3–6.** Spore wall with three layers in young and mature spore (SWL1, SWL2 and SWL3). [3, 4. Young spores with inner layer (SWL3) less pigmented than outer layers (SWL1 and SWL2). 5, 6. Mature spores with inner layer (SWL3) more pigmented than outer layers (SWL1 and SWL2)] **7, 8.** Subtending hyphae slightly funnel-shaped to slightly constricted and all three spore wall layers continuous with subtending hyphal wall (SW). **9, 10.** Detail of septum formed by sublaminae of laminated inner layer (SWL3) typical of *Septoglomus*.

Taxonomic analyses

Septoglomus titan B.T. Goto & G.A. Silva, **sp. nov.** FIGS 2–10

MYCOBANK MB *564321*

Differs from *Septoglomus africanum, S. deserticola,* and *S. xanthium* by its larger spores with 3-layered walls; and from *S. constrictum* by its thicker 3-layered spore walls.

TYPE: BRAZIL: Bahia, near Ourolândia, in soil cultivated with *Agave sisalana*, 20.Apr.2011, B.T. Goto, permanent PVLG slides (**Holotype**, URM83528; GenBank JQ312667, JQ312668, JQ312669. **Isotypes**, URM83529; URM83530; Z+ZT Myc 15119).

ETYMOLOGY: *titan* = in reference to the large spores formed by the new species.

GLOMEROSPORES are yellow brown to orange brown when young to dark red brown at maturity, formed terminally on hyphae, and globose to sub-globose (243–)265–375(–400) µm or (rarely) oblong to irregular, (225–)245–285 × 308–325(–400) µm.

Spore wall is (15.3–)17.9–23.0(–25.6) µm thick in total, comprising three smooth layers (SWL1–3). SWL1 is sub-hyaline to light yellow, thin (0.5–1.4 µm) observed in young and mature spores or in the subtending hyphal wall. SWL2 is pigmented, yellow brown to orange brown, unit (2.5–5.1 µm); darker than SWL3 in young spores, SWL2 acquires pigmentation during maturation. The SWL3 is dark yellow to yellow brown in young spores becoming orange brown to dark red brown in mature spores, laminated,12.8–19.2 µm thick. Spore wall layers are continuous with the subtending hyphal wall layers, and the SWL2 and SWL3 pigments continue into the subtending hyphal wall. The three layers do not stain when exposed to Melzer's reagent.

Subtending hypha (sh) generally present, single, straight, cylindrical to constricted, light yellow to orange brown, 17.9–33.2 µm wide (mean = 25.4 µm) at the spore base. The subtending hyphal wall is 7.6–8.9(–10.2) µm thick (mean 8.2 µm) near the spore base, tapering to approximately 2.5–5.2 µm about 100 µm distant from the spore base; occlusion formed by bridging septum arising from sublaminae of SWL3, although the pore at the subtending hypha sometimes appears partly open.

Germination by regrowth of the subtending hypha.

Glomerospore development deduced from spores found in different developmental stages. The sub-hyaline hyphal wall layer differentiates into sub-hyaline to yellow, evanescent (SWL1) and unified (SWL2) spore wall layers after which the laminate layer 3 differentiates with increasing number of developing sublaminae and becomes pigmented. Finally, the pore is not closed by introverted thickening of SWL3 but by a bridging septum arising from the laminate wall layer at the spore base.

Arbuscular mycorrhiza formation associated with roots of *Sorghum bicolor* (L.) Moench.

Distribution — Currently known only from agricultural soils mainly cultivated with *Agave sisalana* Perrine (*Agavaceae*) in Ourolândia and São Domingos, Bahia State (Brazil).

Additional collections examined: **BRAZIL: Bahia**, near São Domingos, in soil cultivated with *Agave sisalana*, UFRN1895 UFRN1896 UFRN1897.

Discussion

Sequence analyses of the partial LSU rRNA region confirm with high support that the new species represents a *Septoglomus* that differs from the four other described *Septoglomus* species (Fig. 1). The morphology of the subtending hyphal structure (Figs 3–8) also supports the new species in *Septoglomus* as circumscribed by Oehl et al (2011a).

Septoglomus africanum (Błaszk. & Kovács) Sieverd. et al., *S. deserticola* (Trappe et al.) G.A. Silva et al., and *S. xanthium* (Błaszk. et al.) G.A. Silva et al.

differ from *S. titan* by their smaller spores with 2-layered walls [*S. africanum* (60–)87(–125) µm (Błaszkowski et al. 2010); *S. deserticola* 47–54(–115) × 38–52(–102) µm (Trappe et al. 1984); and *S. xanthium* 20–55 × 45–100 µm (Błaszkowski et al. 2004)]. Although *Septoglomus constrictum* (Trappe) Sieverd. et al. also has large (150–330 µm) spores, it differs from *S. titan* by its thinner, 2-layered spore walls with a total thickness of 7–15 µm (Trappe 1977, Oehl et al. 2011a).

Septoglomus titan is easily distinguished from all other glomoid non-*Septoglomus* species by spore wall structure. A few dark species such as *Glomus tenebrosum* (Thaxt.) S.M. Berch and *Funneliformis geosporus* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler resemble *S. titan*. *Glomus tenebrosum* has spores with only two spore wall layers (Thaxter 1922, Trappe 1977, Walker 1982). *Funneliformis geosporus* has spores with three wall layers, but the inner layer is flexible and thin (<1.0 µm), whereas in *S. titan* the SWL3 is laminated and thick.

Goto et al. (2012) noted that the spore wall of *G. trufemii* B.T. Goto et al. was one of the thickest (7.4–15.5 µm) among the glomoid-spored glomeromycotan species, but *S. titan* has even thicker spore walls.

Acknowledgements

The authors acknowledge Dr. Janusz Błaszkowski (Department of Plant Protection, West Pomeranian University of Technology, Szczecin, Poland) and Dr. Fritz Oehl (Agroscope Reckenholz-Tänikon ART, Zurich, Switzerland) for reviewing the manuscript and making helpful comments and suggestions and appreciate the corrections by Dr. Shaun Pennycook (Nomenclature Editor) and suggestions by Dr. Lorelei Norvell (Editor-in-Chief). This work was supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through Protax (Programa de Capacitação em Taxonomia); PPBio (Programa de Biodiversidade do Semiárido) and INCT-HV (Herbário Virtual da Flora e dos Fungos); FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco); UFRN (Programa de Pós-Graduação em Sistemática e Evolução); UFPE (Programa de Pós-Graduação em Biologia de Fungos) and CAPES-PNPD (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Programa Nacional de Pós-Doutorado). The Graduate Program in Microbiologia Agrícola da Universidade Federal do Recôncavo da Bahia is acknowledged for providing technical support.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.111

Volume 124, pp. 111–115 *April–June 2013*

Geoglossaceous fungi in Slovakia 5. *Geoglossum uliginosum*: taxonomy and nomenclature

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Abstract — *Geoglossum uliginosum* is a new record for Slovakia and second record for Central Europe. *Geoglossum glabrum* var. *uliginosum* is a forgotten and disused name representing a different fungus with unknown circumscription. Recent collections of *G. uliginosum* from Slovakia are described and compared with the Swedish holotype and the Czech collections, and the nomenclature and distribution of the species are discussed.

Key words — *Geoglossaceae*, Scandinavia, conservation

Introduction

During field research of geoglossaceous fungi in non-forested stands in Slovakia, an interesting species of *Geoglossum* was collected and identified as *Geoglossum uliginosum*. Although collections from the Czech Republic have already been published (Kučera & Gaisler 2012), the Slovak specimens were collected and identified first. *Geoglossum* species are rare in Slovakia, and only a few taxa had been reported before 2000 when we started our research (Kučera et al. 2008, 2010, 2012; Kučera 2012). Geoglossaceous fungi, which have been used in studies evaluating European grasslands (Rald 1985; Nitare 1988; Jordal 1997; McHugh et al. 2001; Adamčík & Kautmanová 2005), are of conservation interest. In Sweden, *Geoglossum uliginosum* is treated in a special 'action plan' for nature conservation (Nitare 2007).

Materials & methods

Macromorphological characters were observed in fresh material. The micromorphological structures were observed in dried material using a light microscope

with an oil immersion lens. Fragments of fruit-bodies were examined in tap water, 5% KOH, Melzer's reagent, and a solution of Congo red in ammonia. Quantitative values for micromorphological characters are presented as mean ± 1 standard deviation of 30 measurements for each character (with minimum/maximum values in parentheses). Herbarium acronyms are in accordance with Index Herbariorum (Thiers 2013). Voucher specimens were deposited in herbaria PRM, BRNM, SAV, and UPS. Localities are georeferenced and the coordinates are given in the WGS 84 system. The description is based on collections from Slovakia.

Description of studied Slovak collections

Geoglossum uliginosum Hakelier, Svensk Bot. Tidskr. 61: 422, 1967.

Ascocarps (35–)38–58(–66) mm high, clavate, stipitate, scattered, solitary. Fertile part (10–)14–22(–25) × (2–)3–6(–8) mm, usually flattened, lanceolate, black, occasionally vertically grooved, glabrous. Sterile part 20–40(–50) × 1–3 mm, clearly delimited from the fertile part, cylindrical, black, smooth, viscid when fresh, ± shiny after drying [22 fruitbodies examined]. Asci (143–)156–189(–214) × (11–)15–20(–24) µm, clavate, apex rounded, narrowed, 8-spored, pore blued in Melzer's reagent. Spores (48–)59–75(–80) × 4–5(–6) µm, cylindrical, slightly curved, tapering towards one end, dark fuliginous, in one cluster in the upper part of the ascus, 7-septate, occasionally 5- or 6-septate. Paraphyses slightly protruding above the asci, fragile, brownish in the apical part, ≤9 µm diam, remotely septate in the basal portion, closely septate in the apical part, constricted at the septa. The constrictions occur at every second septum. The cell pairs form chains and are easily broken off at the constrictions. One of the cell pairs may be inflated to pyriform or globular form, ≤6–8 µm diam, especially in the apical part of the paraphyses. Instructive drawings of paraphyses (and asci and spores) taken from the holotype specimen were published by Nitare (2007).

Habitat — Peat-bogs and wet meadows in association with *Sphagnum* sp., *Eriophorum* sp., *Deschampsia caespitosa, Nardus* sp., *Molinia caerulea,* and various species of *Carex.*

Distribution — Czech Republic (Kučera & Gaisler 2012), Norway (Fadnes 2008; Nitare 2007), Slovakia (this paper), Sweden (Hakelier 1967; Nitare 1984, 2007; Ohenoja 2000, Turander 2012). Reports from Scotland and Northern Ireland probably relate to a different species (Nitare 2007).

Specimens studied: **SWEDEN**, **Västmanland**, Viker parish, Älvhyttan, Venen, 11.9.1965, N. Hakelier (holotype, UPS BOT: F-013939). **SLOVAKIA**, **Banská Bystrica**, Poľana Mts., Hriňová, near mountain hotel "Poľana", along the trail to the top of the Poľana hill, 48°37′39.73″N 19°28′01″E, alt. 1304 m, wet meadow with *Deschampsia caespitosa, Eriophorum* sp., *Sphagnum* sp. 27.9.2009, V. Kučera (SAV F–10162); 6.10.2011, V. Kučera (SAV F–10529).

Discussion

Nomenclature — The name *Geoglossum uliginosum* could potentially be confused with *G. glabrum* [var.] β *uliginosum* Pers. (Persoon 1800: 62, tab. 3 fig. 4). As Persoon's name was apparently never raised to specific rank, however, Hakelier's name is legitimate and not in conflict. *G. glabrum* var. *uliginosum* certainly represents a different taxon because it was described as having a squamulose stipe, while that of *G. uliginosum* is completely smooth. We were unable to locate any specimen labelled either *Geoglossum glabrum* var./f. *uliginosum* or *Geoglossum uliginosum* in Persoon's herbarium (L), and identification of Persoon's fungus based only on the original (sparse) description is impossible.

Taxonomy — *Geoglossum uliginosum* is characterised by having 1) ascocarps with a slightly slimy-viscid stipe and 2) paraphyses with characteristically long and easily broken chains of cells (or cell pairs) that are swollen at one or sometimes both ends. The species grows only in slightly wet grasslands among *Sphagnum*. The material from Central Europe (both from Slovak and Czech Rpublics) does not differ in any essential character from the holotype and other Swedish collections; minor differences are presented in Table 1.

Table 1. Comparison of asci and ascospores in *Geoglossum uliginosum* from different countries.

Country	Asci (µm)		Ascospores (µm)		No. of septa
	Length	Width	Length	Width	
Slovakia	(143–)156–189(–214)	(11–)15–20(–24)	(48–)59–75(–80)	4–5(–6)	(5–)7
Czech Republic	(151–)167–190(–196)	(13–)15–19(–24)	(50–)63–78(–90)	5–7	(5–)7(–9)
Sweden	140–175	14.5–17	60–80	4.5–6	7

Specimen data: Slovakia — SAV F-10162, SAV F-10529; Czech Republic — PRM 860478, PRM 860479, PRM 860480, BRNM 737698, BRNM 737699, SAV F-10531, SAV F-10532 (see Kučera & Gaisler 2012); Sweden — Holotype UPS BOT F-013939 (Hakelier 1967).

Conservation — *Geoglossum uliginosum* is well known from Scandinavia (Sweden and Norway) and it was assumed that its distribution is restricted to slightly wet and unfertilised semi-natural grasslands in the hemiboreal and southern boreal vegetation zons in Scandinavia (Nitare 2007). Records from Central Europe are therefore noteworthy.

We expect to find *Geoglossum uliginosum* in other locations in Central Europe with habitats similar to those in Poľana and Jizerské hory mountains. The species is apparently rare and should be included in the next edition of the Red list of Slovakia and Red list of Czech Republic. Currently the species is red-listed in Sweden (Gärdenfors 2010).

Semi-natural grasslands are valuable landscape elements with high diversity of plant and animal life and represent a reservoir of indigenous biodiversity. Such habitats are critically threatened all over Europe, their number decreasing mainly due to the fertilization, lack of grazing animals, and industrial pollution (Newton et al. 2003).

Acknowledgements

We would like to thank Paul Cannon (CABI and Royal Botanic Gardens, Kew, UK) and Esteri Ohenoja (University of Oulu, Finland), who read and commented on the manuscript, and Shaun Pennycook (Manaaki Whenua Landcare Research, Auckland, New Zealand) for his support. Václav Kautman, Stanislav Glejdura, and Nikola Rybáriková are acknowledged for their assistance in the field, and Gerard Thijsse and the staff of the Nationaal Herbarium Nederland in Leiden for the opportunity to study Persoon's herbarium. This study was supported by grant VEGA 2/0062/10 to VK and PL and grant MZE 0002700604 to JG.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.117

Volume 124, pp. 117–126 *April–June 2013*

A new endophytic species of *Chaetomium* from *Jatropha podagrica*

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Abstract — A new endophytic species of *Chaetomium* from fruits of *Jatropha podagrica* is described from Maharashtra State, India. A combined sequence dataset of the ITS region, LSU rDNA, and β-tubulin genes supports recognition of this fungus as a new species that is largely concordant with morphological characters. *Chaetomium jatrophae* is characterized by spirally coiled, predominantly unbranched, and coarsely roughened terminal ascomatal hairs and elongate-ellipsoidal ascospores with a subapical or lateral germ pore. It is a mesophilic *Chaetomium* species with a temperature maximum of 40±0.2°C.

Key words — *Chaetomiaceae*, *Euphorbiaceae*, phylogeny, taxonomy

Introduction

Chaetomium Kunze (Kunze 1817) (*Chaetomiaceae*) accommodates more than a hundred species. *Chaetomium* species are widespread and found on various substrates, most with remarkable cellulolytic activity. Some are pathogenic for humans (Barron et al. 2003, Al-Aidaroos et al. 2007, de Hoog et al. 2009, Hubka et al. 2011) and others are plant endophytes (Syed et al. 2009). *Chaetomium* is a widely studied genus (Ames 1969; von Arx et al. 1986; Gené & Guarro 1996; Decock & Hennebert 1997; Udagawa et al. 1997; Rodríguez et al. 2002; Wang & Zheng 2005a,b; Asgari & Zare 2011) that is mainly characterized by superficial ostiolate ascomata covered with hairs. During our biodiversity survey of endophytic fungi, we isolated 20 strains of fungi from *Jatropha podagrica* belonging to 13 species. Two strains belonged to *Chaetomium: C. bostrychodes* Zopf (MCC 1019), isolated from a leaf petiole, and a new species isolated from the fruit of the same plant. This new species is described here as *Chaetomium jatrophae* based on morphological and combined phylogenetic data of three loci.

Table 1. *Achaetomium* and *Chaetomium* isolates used in phylogenetic analysis.

Taxon	Strain	GenBank accession numbers		
		ITS	LSU	β-tubulin
A. strumarium	CBS 333.67 (T)	AY681204	AY681170	AY681238
C. acropullum	CBS 126783	HM365258	HM365258	HM365292
C. ancistrocladum	CBS 126784	HM365241	HM365241	HM365290
	CBS 126673	HM365242	HM365242	HM365291
C. atrobrunneum	CBS 379.66 (T)	HM365255	HM365255	HM365294
C. bostrychodes	IMI 325809	HM365261	HM365261	HM365296
C. carinthiacum	CBS 126669	HM365265	HM365265	HM365299
C. coarctatum	CBS 162.62 (T)	HM365260	HM365260	HM365281
C. crispatum	CBS 152.49	HM365267	HM365267	HM365293
C. cruentum	CBS 371.66 (T)	HM365266	HM365266	HM365270
C. elatum	CBS 126657	HM365236	HM365236	HM365282
	CBS 126656	HM365238	HM365238	HM365284
	CBS 910.70 (T of *C. ramipilosum*)	HM365237	HM365237	HM365283
C. funicola	IMI 300511	HM365262	HM365262	HM365300
C. globosum	CBS 148.51	HM365254	HM365254	HM365269
C. grande	CBS 126780 (T)	HM365253	HM365253	HM365273
C. indicum	CBS 126667	HM365248	HM365248	HM365301
	CBS 126668	HM365249	HM365249	HM365302
C. interruptum	CBS 126661	HM365245	HM365245	HM365276
	CBS 126662	HM365244	HM365244	HM365275
	CBS 126660 (T)	HM365246	HM365246	HM365277
C. iranianum	CBS 126670 (T)	HM365257	HM365257	HM365297
C. jatrophae	**MCC 1025, CBS 134263 (T)**	**JQ246354**	**HE981193**	**HE981190**
C. jodhpurense	CBS 280.79	HM365243	HM365243	HM365295
C. madrasense	CBS 126663	HM365252	HM365252	HM365274
C. megalocarpum	CBS 533.79	HM365259	HM365259	HM365271
	CBS 126666	HM365264	HM365264	HM365272
C. murorum	CBS 126785	HM365256	HM365256	HM365288
	CBS 636.83	HM365268	HM365268	HM365289
C. rectangulare	CBS 126659	HM365240	HM365240	HM365286
	CBS 126778 (T)	HM365239	HM365239	HM365285
C. subaffine	CBS 126777	HM365247	HM365247	HM365280
C. truncatulum	CBS 126782 (T)	HM365263	HM365263	HM365298
C. undulatulum	CBS 126776	HM365250	HM365250	HM365278
	CBS 126775 (T)	HM365251	HM365251	HM365279

Sequences in bold font were generated in this study. (T) = ex-type strain.
CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
IMI = CABI BioScience, Egham, UK.

Materials & methods

Sampling, fungal isolation and growth

The fungus was isolated from a healthy fruit of *Jatropha podagrica*, an ornamental plant, collected from Pune (Pimpri), Maharashtra, India (18°37′07.04″N 73°48′13.43″E). Various plant parts (leaves, petioles, fruits, flowers, young stems) were used to isolate various endophytic fungi upon Potato Dextrose Agar (PDA) and Czapek Dox Agar (CDA). Healthy fruits were harvested with clean blades and immediately dipped in beakers containing water. The fruits were subsequently washed with tap water (10 min), cut into fragments, washed twice in sterile distilled water (5 min), 70% ethanol (2–3 min), and 100% ethanol (20–30 sec). The fungal strains were isolated on CDA at room temperature after 3 d of incubation. Colony growth rate and characteristics were recorded on 2% MEA (Hi-Media, India) at room temperature (28–30°C) after 7 d in dark. Growth rate was also measured at different temperatures (20–45°C, at intervals of 5±0.2°C) on five media including PDA, CDA (Hi-Media, India), PCA (Potato Carrot Agar), MEA (Malt Extract Agar) and OA (Oatmeal Agar) after 7 d.

Morphological observations and scanning electron microscopy (SEM)

The ascomata and ascospores were studied on slides mounted in water or lactophenol. The slides were observed under Nikon YS100 microscope (Nikon, Japan). Microphotographs were taken on Olympus BX53 (Olympus Corporation, Japan) fitted with ProgRes C5 camera (Jenoptik, USA). Measurements of morpho-taxonomic characters were recorded and compared with type descriptions of known species (Ames 1969, Tiwari et al. 1977, Wang & Zheng 2005a,b; Asgari & Zare 2011). Fifty readings were recorded for each measurement using Jenoptik software. Ascomatal hair ornamentation was determined by scanning electron microscopy (SEM). Dry ascomata were placed on black double-sided tape on a stub (1 cm diam.), coated with platinum in a Jeol sputter coater (Jeol-JFC 1600), and then visualized in SEM (Jeol-JSM 6360A) at 10 kV.

DNA extraction

Genomic DNA for amplification of internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) was extracted using QIAamp® DNA Mini Kit (Qiagen, Inc., Valencia CA). Genomic DNA for D1, D2 region of 28S large subunit (LSU) of ribosome and β-tubulin (BTUB) genes was extracted by crushing fungal mycelia in cetyl trimethylammonium bromide (CTAB) extraction buffer, pH 8 (Hi-Media, India). Thereafter 20 µl of proteinase K (20 mg/ml) was added and incubated at 65±0.2°C for 1 h (Baker & Mullin 1994), followed by addition of 20 µl RNase (10 mg/ml) and further incubation at 65±0.2°C for 15 min. Chloroform: isoamylalcohol (24:1) was added (500 µl) to the supernatant collected after centrifugation (8000 rpm, 10 min). The mixture was shaken for 5 min and centrifuged at 10,000 rpm, 4°C for 15 min. Two volumes of CTAB precipitation buffer (1% CTAB, 50 mM Tris, pH 8.0, 10 mM EDTA) was added to the supernatant and kept at room temperature for 1 h. The pellet collected after centrifugation was dissolved in 500 µl of 1.2 M NaCl and then 500 µl of chloroform: isoamylalcohol (24:1) was added. Two volumes of absolute alcohol were added to the aqueous phase to precipitate the DNA (Murray & Thompson 1980, Brandfass & Karlovsky 2008). The quantity of DNA was measured by the amount of absorbance of the sample at 260 nm on NanoDrop (ND-1000, Thermo Scientific, USA) and purity was checked on 0.8% agarose gel electrophoresis.

PCR amplification and DNA sequencing

The ITS-LSU rDNA region was amplified using the 5.8S (CGCTGCGTTCTTCATCG) and LR7 (TACTACCACCAAGATCT) primers (http://www.botany.duke.edu/fungi/mycolab/primers.htm). The reaction mixture (25 µl) containing genomic DNA (100 ng), 1X PCR buffer, 0.2 mM dNTPs, 10–20 pmoles of each primer, and 1 unit of Taq DNA polymerase was amplified with Gene Amplifier PCR System 9700 (Perkin Elmer, USA) using the following protocol: initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 1 min, 57°C for 30 sec, 72°C for 2 min, and final extension at 72°C for 10 min. The BTUB gene was amplified using primers T1 (AACATGCGTGAGATTGTAAGT) and Bt2b (ACCCTCAGTG TAGTGACCCTTGGC) (O'Donnell & Cigelnik 1997) using the same reaction mixture and equipment as for the ITS-LSU region using the following protocol: initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 30 sec, and final extension at 72°C for 10 min. Five µL of the PCR amplified product was run on 1.0% agarose gel in 1X TBE buffer (54 g Tris base, 27.5 g boric acid, 20 ml EDTA 0.5 M, pH 8). Electrophoresis was carried out for about 90 min at 80 V. The gel was stained in 1% ethidium bromide for 15 min and observed under UV illumination. The PCR products were purified according to Sambrook et al. (1989). The sequencing reactions were carried out using the 'ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit' (Perkin Elmer Applied Biosystems Division, Foster City, CA) according to the manufacturer's protocol. DNA sequencing was carried out on ABI 3730xl Automated Sequencer.

Phylogenetic analysis

An NCBI and DDBJ BLASTn search for ITS region (555 bp) was conducted for sequence similarity (Zhang et al. 2000). The phylogenetic relationships of *C. jatrophae* and other *Chaetomium* species were examined using ITS, LSU, and BTUB gene sequences. Thirty-three sequences corresponding to 23 *Chaetomium* species were retrieved from GenBank along with the outgroup, *Achaetomium strumarium* (TABLE 1). The sequences generated in this study were manually edited using ChromasPro version 1.34. The ITS region, rDNA LSU, and BTUB gene sequences were analyzed individually and together in MEGA 5.0. All sequences were aligned by multiple alignments using MUSCLE program in MEGA 5.0 (Edgar 2004, Tamura et al. 2011). Sequences were analyzed by maximum parsimony (MP) statistical method using bootstrap method of phylogeny test (1000 replicates and 10 initial trees) (Tamura et al. 2011). Gaps and missing data were deleted. MP tree was obtained by close-neighbor-interchange (CNI) on random trees algorithm (Nei & Kumar 2000). Bootstrap values below 50% were removed from tree. Sequences were also analyzed by neighbour- joining (NJ) and maximum likelihood (ML).

Results

Taxonomy

Chaetomium jatrophae Rohit Sharma, **sp. nov.** PLATES 1–2

MYCOBANK 563940

Differs from *Chaetomium atrobrunneum* by its larger ascomata with spiraling coarsely roughened terminal hairs, its peridium of texture intricata, and its ascospores with a sub-apical to lateral germ pore.

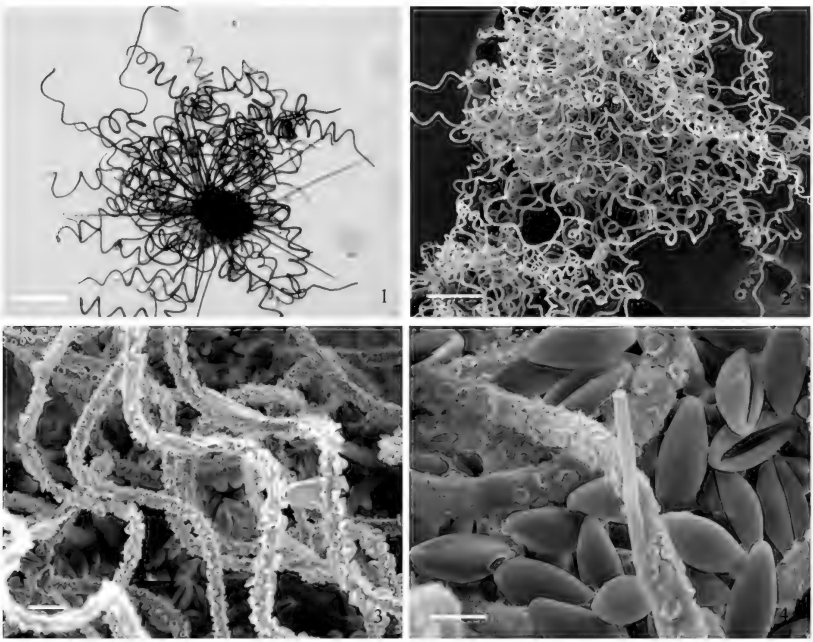

PLATE 1. *Chaetomium jatrophae* (MCC 1025). FIG. 1: Mature ascoma, showing terminal coiled terminal hairs. FIG. 2, 3: Ascomatal hairs (SEM). FIG. 4: Ascospores (SEM). Scale bars: 1 = 100 µm; 2, 3 = 10 µm; 4 = 5 µm.

TYPE: India, Maharashtra State, Pune, Pimpri, 18°37′07.04″N 73°48′13.43″E, endophytic in fruit of *Jatropha podagrica* Hook. (*Euphorbiaceae*), 25.IV.2011, Rohit Sharma (**Holotype**, AMH 9558; ex-type culture, MCC 1025, NFCCI 2596, CBS 134263).

ETYMOLOGY: Refers to the host genus *Jatropha*.

Colonies reaching 60 mm diam. on MEA after 7 d at room temperature (28–30°C), olivaceous-buff to grayish-sepia with sparse aerial mycelium; reverse similar in color to colony surface. Mycelium composed of hyaline to subhyaline, septate, smooth hyphae, 9.0–9.4 µm diam. Ascomata superficial to immersed, scattered, ostiolate, globose to subglobose, 187.3 (161.1–193.8) × 151.6 (129.2–161.1) µm, brown to dark brown, maturing in 20 d, loosely attached to mycelium with brown to olivaceous brown rhizoids. Peridium brown-vinaceous to brown-olivaceous, membranaceous with textura intricata. Terminal hairs of different length, 312.5 (250.0–375.0) µm long, numerous, pale yellowish-brown to dark brown, straight below and spirally coiled above, 8–11 coils (generally 7–8), 3.9 (3.2–4.8) µm wide at the base gradually tapering and paling towards the apex, hairs forming dense tuft around ostiole, unbranched, rarely branched, thick walled, coarsely roughened, septate. Lateral hairs straight, short, coarsely roughened, 56.0 (32.3–83.9) µm in length and 3.5 (3.2–4.0) µm

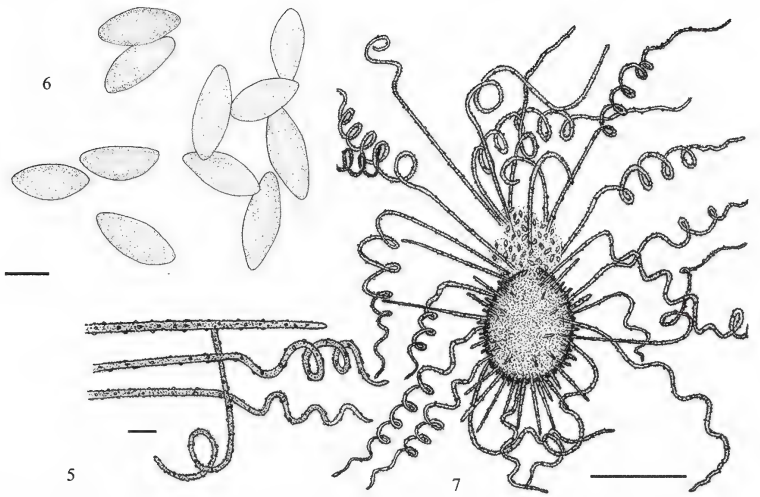

Plate 2. *Chaetomium jatrophae* (MCC 1025). Fig. 5: Ascomatal hairs. Fig. 6: Ascospores. Fig. 7: Mature ascomata. Scale bars: 5, 6 = 5 µm, 7 = 150 µm.

wide at the base. Paraphyses absent. Asci clavate, hyaline, evanescent. Ascospores one-celled, brown when mature, fusiform or elongate-ellipsoidal, symmetrical or sometimes asymmetrical, 12.3 (6.5–16.2) × 7.6 (6.5–9.7) µm, thick-walled, with a distinct sub-apical or lateral germ pore. Anamorph absent.

Cultural characteristics

The optimum growth of *C. jatrophae* was determined as 30±0.2°C (mesophilic in nature) and its temperature maximum at 40±0.2°C (Fig. 8). The temperature

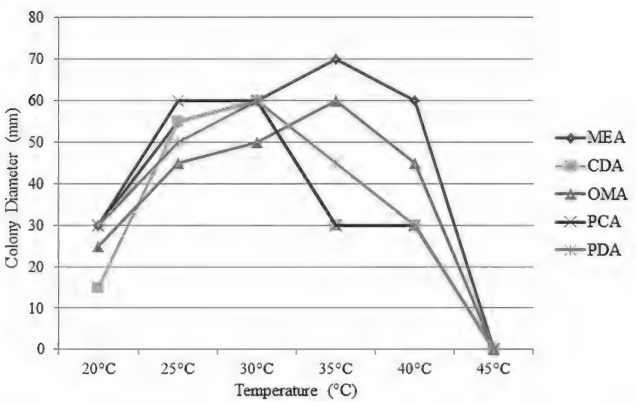

Fig. 8. Temperature-growth relationship of three *Chaetomium jatrophae* stains on Malt Extract Agar (MEA), Czapek Dox Agar (CDA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), and Potato Carrot Agar (PCA) (mm diam. of 7 d old colonies).

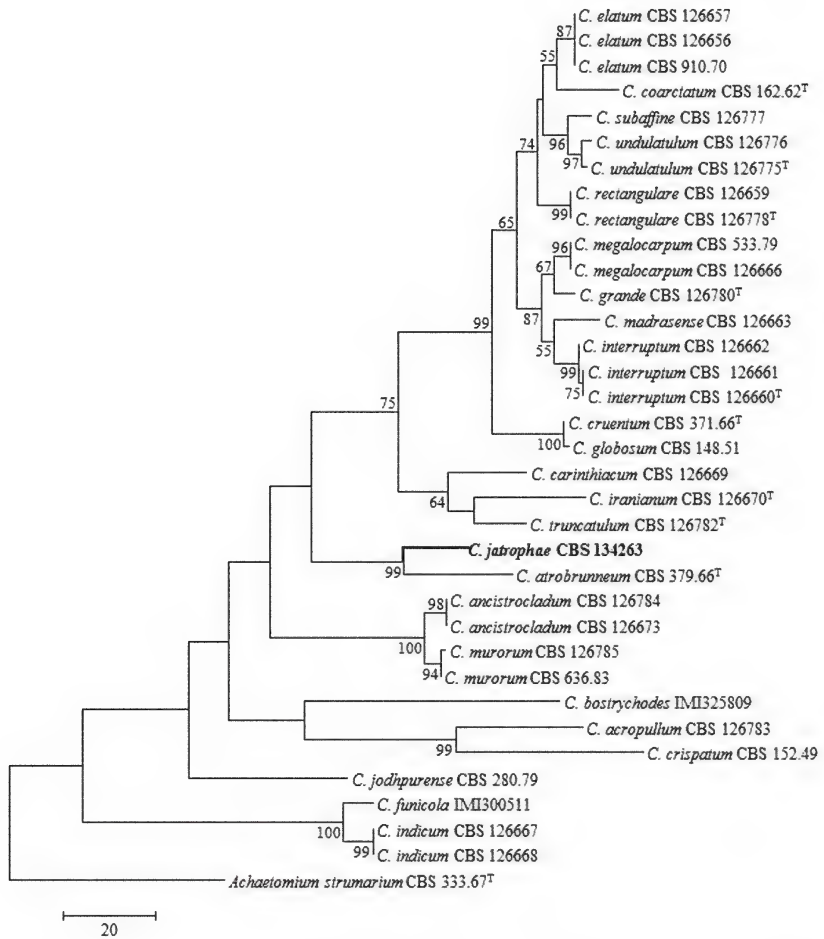

FIG. 9. Maximum parsimony phylogram of *Chaetomium jatrophae* (MCC 1025) based on combined dataset of ITS–LSU rDNA–BTUB gene sequences, with *Achaetomium strumarium* as outgroup. Bootstrap values > 50% (1000 replicates) are shown; CI = 0.531860, RI = 0.705642, RCI = 0.375302; gaps and missing data deleted. *C.* = *Chaetomium*, (T) = ex-type strain.

maximum is an important physiological character for species differentiation (Li et al. 2012). The maximum fungal growth occurred primarily on MEA followed by OA. Ascomata developed after 25–30 d of incubation on MEA.

Molecular analyses

The phylogenetic analysis of 24 taxa of *Chaetomium* was conducted using ITS and LSU (D1, D2 region) of rDNA and BTUB gene and sequence data

analyzed individually (not shown) and in combination. The ITS alignment consisted of 576 bases (438 conserved, 131 variable, 91 parsimony informative, 40 singleton). The maximum parsimony tree had CI = 0.687500 RI = 0.805654 and RCI = 0.553887 (for all sites). The LSU alignment consisted of 386 bases (361 conserved, 25 variable, 22 parsimony informative, 3 singleton), and the BTUB gene alignment consisted of 425 bases (209 were conserved, 215 variable, 193 parsimony informative, 22 singleton). The combined data set had 1392 sites (1008 conserved, 371 variable, 306 parsimony informative, 65 singleton). One most parsimonious tree is shown in Fig. 9. All phylogenetic analyses (NJ, MP, ML) supported the novelty of this species.

Discussion

The newly described species, *C. jatrophae*, is morphologically and molecularly differentiated from related species. Maximum parsimony analysis of combined dataset of ITS-LSU rDNA-BTUB gene (Fig. 9) groups *C. jatrophae* in a clade with *C. atrobrunneum* L.M. Ames with 97% bootstrap support. Although the LSU and BTUB sequence data is unavailable for *C. jabalpurense* D.P. Tiwari et al., the ITS sequence analysis (not shown) distinctly separates *C. jatrophae* from the morphologically similar *C. jabalpurense* and *C. perlucidum* Sergeeva. *Chaetomium jatrophae* resembles *C. jabalpurense*, *C. atrobrunneum,* and *C. perlucidum* mainly by ascospore shape and germ pore. Nevertheless, *C. jabalpurense* (Tiwari et al. 1977) can also be distinguished from *C. jatrophae* by its larger ascomata (154.7–304.5 × 150.3–297.2 µm), narrower (3.2 µm) terminal hairs, spirally coiled lateral hairs, and narrower (5.3–6.2 µm diam.) ascospores. *Chaetomium atrobrunneum* (Ames 1949), also close to *C. jatrophae*, is distinguished mainly by smaller ascomata (75–120 µm), peridium of textura angularis, straight seta-like mostly smooth-walled ascomatal hairs, and fusiform or elongate pyriform ascospores with a slightly subapical germ pore. *Chaetomium perlucidum* (Ames 1969) is differentiated from *C. jatrophae* by its olive-yellow to pale-brown smaller (105–135 × 100–130 µm) ascomata and narrower (5.6–6 µm diam.) ascospores.

The fact that *Chaetomium jatrophae* was isolated from *Jatropha podagrica,* a xerophytic latex-producing plant may help fungi to survive better than in outside environment. The occurrence of this *Chaetomium* species in fruits suggests that further research is needed to better understand its biology as well as its obligatory or facultative endophytic nature, and its role as an endophyte in *J. podagrica.* However, Syed et al. (2009) pointed that endophytic *Chaetomium* species are similar to *Chaetomium* strains obtained from other substrates (e.g., scat, seed, soil) both physiologically (utilizing the same carbon source) and genetically (similar ITS rDNA sequence data).

Acknowledgments

The authors are grateful to DBT, India for funding, NCCS, India for laboratory facilities and Dr. S.V. Shinde (University of Pune, India) for SEM microscopy. We are also indebted to Dr. Xue-Wei Wang (State Key Laboratory of Mycology, China) and Dr. Yei-Zeng Wang (National Museum of Natural Science, Taiwan) for their assistance during preparation of manuscript. Dr. Bita Asgari (Iranian Research Institute of Plant Protection, Iran) and Dr. Vit Hubka (Charles University, Czech Republic) are also acknowledged for critical review of the manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.127

Volume 124, pp. 127–141 *April–June 2013*

A contribution to the taxonomy of *Lyromma* (*Lyrommataceae*, lichenized *Ascomycota*) with a species key

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Abstract — The paper presents a new concept of conidial-ascosporic relationship in the foliicolous lichenized genus *Lyromma* and a revised key to species determination. *Lyromma coronatum* sp. nov. (Bolivia) is characterized by reduced perithecial appendages composed of clavate hyphae that form a crown around the ostiole. *Lyromma multisetulatum* sp. nov. (Bolivia, Brazil) is characterized by numerous large strongly recurved perithecial appendages composed of individual acicular hyphae. Undescribed ascosporic states for *Lyromma dolicobelum* and *L. palmae* and conidial states for *L. ornatum* and *L. pilosum* are described based on observations of perithecia and pycnidia for the first time in natural conditions. Confirmation of a conidial-ascosporic connection for *L. nectandrae* suggests *L. confusum* as a synonym. *Lyromma dolicobelum*, *L. nectandrae*, and *L. ornatum* are reported as new to Bolivia, and *L. pilosum* as new to Bolivia and Brazil.

Key words —Neotropics, new species, South America

Introduction

This is a new contribution to the foliicolous lichens of Bolivia (Flakus & Lücking 2008) within an inventory aimed to establish knowledge on the diversity of lichens and allied fungi in the country (e.g. Flakus & Wilk 2006, Flakus et al. 2008, Flakus 2009, Knudsen & Flakus 2009, Kukwa & Flakus 2009, Flakus & Kukwa 2012, Flakus et al. 2012, Kukwa et al. 2012).

The foliicolous lichenized genus *Lyromma* Bat. & H. Maia (*Lyrommataceae* Lücking, *Chaetothyriales*), containing six species accepted by Lücking (2008), is characterized by globose to short barrel-shaped perithecia and elongate barrel-shaped pycnidia, sessile on smooth ecorticate thalli of rounded patches formed by symbiotic relationship with *Phycopeltis*. Perithecia are composed of a thin

layer of irregular dark brown hyphae, and persistent or evanescent paraphyses comprise the hamathecium. The bitunicate obclavate to saccate asci (I–, KI–) contain 8 transversely septate fusiform colorless ascospores. Pycnidia (formed by a thin layer of distinctly parallel dark brown hyphae) produce multiseptate filiform colourless macroconidia or non-septate bacilliform microconidia. Mature perithecia and pycnidia both form appendages in the upper part near ostiole, composed of either individual or agglutinated hyphae (Batista & Maia 1965, Cavalcante et al. 1972, Lücking 1992, 2008, Lücking et al. 1998).

Lyromma was established as a monospecific pycnidial genus for *L. nectandrae* (Batista & Maia 1965, Lücking et al. 1998). Perithecia were described originally for *L. nectandrae* (Lücking 1992, Aptroot et al. 1997) and later for *L. ornatum* (Lücking & Kalb 2000). For a long time, *L. nectandrae* was the only species known to produce both perithecia and pycnidia (Lücking 1992, Aptroot et al. 1997). However, a re-examination of the known *Lyromma* material by Lücking (2008) showed that no single collection contained both perithecia and pycnidia on the same thallus. Moreover, specimens assigned to *L. nectandrae* with perithecia were shown to belong to different taxa described later as *L. confusum* and *L. pilosum* (Lücking 2008). Since ascosporic and conidial states were not observed on the same thallus, the perithecial and pycnidial morphotypes had been treated as different species until their taxonomic status could be clarified. Finally, based on the then-available specimens, Lücking (2008) proposed three taxa with perithecia and three with pycnidia.

We have been able to study further fresh collections of foliicolous lichens from Bolivia and Brazil and discovered more and more thalli of the otherwise rare *Lyromma* species. These new findings form the basis of our re-evaluation of the species concept based on conidial-ascosporic relationships.

Material & methods

Morphology and anatomy were examined by using Nikon SMZ800 and a Nikon Eclipse 80i (DIC), as well as Olympus SZX9 and Olympus BX50 (DIC) microscopes. Preparations were studied in water and KOH solution (K), and for ascus structure in Lugol's iodine solution without (I) or with pre-treatment with KOH (K/I). All anatomical measurements were made in water.

Handling the sporocarps required great care to establish exact sizes and numbers of setae. Anatomy, measurements, and number of setae should be examined on sectioned or squash mounts in water under a compound research microscope.

Results

Lyromma coronatum Flakus & Farkas, **sp. nov.** Plate 1a, b

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Differs from other *Lyromma* species by its reduced perithecial appendages composed of numerous individual clavate hyphae that form a regular crown around the ostiole.

TYPE — Bolivia, Dept. Beni, Prov. Ballivian, near Río Pircayo, 14°18′56″S 67°26′34″W, 190 m, lowland Amazon forest, 4 Dec. 2004, A. Flakus 4315 (**Holotype**, KRAM; **isotype**, LPB).

ETYMOLOGY — The species is named after its reduced appendages forming a characteristic crown around ostiole.

THALLUS foliicolous, epiphyllous, ecorticate, formed by rounded patches, smooth, 0.4–3 mm wide, olive-green; PHOTOBIONT *Phycopeltis*, cells rectangular, 12–20 × 4–6 µm, arranged in radiate plates; PERITHECIA sessile, globose, 50–100 µm in diam., and 50–80 µm high, pale brown, around ostiole furnished with crown of almost reduced appendages; OSTIOLE apical, 20–35 µm wide; SETAE dark brown, almost reduced to 5–7(–12) × (4–)5–7 µm, forming a very regular crown around ostiole, composed of numerous individual clavate, apically thickened (apical walls c. 1 µm thick) hyphae (sometimes branched apically); PERIDIUM pale brown, 3–5 µm thick, composed of rectangular cells (3–5 µm wide), thick walled (c. 1–1.5 µm thick), arranged irregularly to almost parallel; PARAPHYSES sparse in mature perithecia, simple, c. 1 µm thick; ASCI bitunicate, 8-spored, 30–50 × 10–15 µm; ASCOSPORES fusiform, hyaline, 3-septate, with slight constrictions at septa, upper second cell usually slightly enlarged, 17–20 × 4–5 µm; PYCNIDIA not seen.

DISTRIBUTION & ECOLOGY — The new species is known from two localities in Bolivia, one of which is located in the protected area Reserva Nacional de Vida Silvestre Amazónica Manuripi. It grows on leaves of vascular plants in lowland Amazon forest.

ADDITIONAL SPECIMENS EXAMINED — **BOLIVIA. DEPT. PANDO.** PROV. MANURIPI, Reserva Nacional de Vida Silvestre Amazónica Manuripi, Bajada colony near Chive village, near Río Madre de Dios, 12°24′03″S 68°26′45″W, 170 m, 2006, A. Flakus 6018 (herb. Flakus).

COMMENTS — *Lyromma coronatum* is characterized mainly by pale brown perithecia and the presence of reduced perithecial appendages that form a characteristic crown around the ostiole composed of numerous very short clavate hyphae. The species can be confused with the ascosporic state of *L. palmae*, which produces appendages composed of similar clavate hyphae. Therefore, we initially regarded the new species as the juvenile form of *L. palmae* with not fully developed appendages. However, a detailed study showed that *L. coronatum* is mature species with fully developed asci and the largest ascospores yet known in the *Lyromma* (Lücking 2008). Thus, *L. palmae* can be distinguished from the new species by its darker perithecia (especially in the upper part), and the perithecial appendages that are larger [10–30(–40) µm long], erect to horizontal, composed of about 20–60 individual hyphae, and usually grouped in 3–6 brush-shaped appendages (then faintly agglutinated in basal part). The two species also differ in the size of the photobiont cells (12–20 × 4–6 µm in *L. coronatum*, and 10–13 × 5–6 µm in *L. palmae*), ascospores

(17–20 × 4–5 µm in *L. coronatum,* and 14–18 × 3–5 µm in *L. palmae*), and the ostiole width (25–35 µm in *L. coronatum,* and 15–20 µm in *L. palmae*).

Lyromma dolicobelum Cavalc., in Cavalcante et al., Publ. Inst. Micol. Univ. Fed. Pernambuco 647: 39. 1972. PLATE 1C

TYPE — Brazil. Roraima: Mucajaí-Caracaraí, II 1962, de Lima 19682 (URM 37178 – holotype; INPA– isotype).

"*Lyromma nectandrae* var. *dolicobelum*" Bat. & Cavalc., in Bezerra et al., Atas Inst. Micol. Univ. Fed. Pernambuco 5: 386, 389, 397, 409, 421. 1967, nom. nud.

[See Lücking (2008) for a detailed description of pycnidia.]

THALLUS foliicolous, epiphyllous, ecorticate, formed by rounded patches, smooth, 2–8 mm wide, olive-green; PHOTOBIONT *Phycopeltis*, cells rectangular, 10–17 × 4–7 µm, arranged in radiate plates; PERITHECIA sessile, subglobose to globose, 50–80 µm in diam., and 60–80 µm high, dark brown, around ostiole furnished with 2–3(–4) setae; SETAE dark brown to black, tubular, almost horizontal to recurved, 60–200(–300) µm long and 20–30 µm broad in basal part, composed of agglutinated hyphae; PERIDIUM dark brown, lower part slightly paler, 2–4 µm thick, 3–5 µm thick, composed of rectangular cells (2–5 µm wide), thick walled (c. 1–1.5 µm thick), arrangement irregular to almost parallel; PARAPHYSES sparse in mature perithecia, simple, c. 1 µm thick; ASCI bitunicate, 8-spored, 30–35 × 10–15 µm; ASCOSPORES fusiform, hyaline, 3-septate, with slight constrictions at septa, 16–18 × 4–5 µm.

DISTRIBUTION & ECOLOGY — This species, known previously only from lowland Amazon forest in Brazil and lowland rainforest in Mexico (Cavalcante et al. 1972, Lücking et al. 1998, Herrera-Campos et al. 2004, Lücking 2008), is now reported for Bolivia.

SELECTED SPECIMENS EXAMINED — **BOLIVIA. DEPT. PANDO.** PROV. MANURIPI, Reserva Nacional de Vida Silvestre Amazónica Manuripi, Puerto Rico village, 11°06′12″S 67°31′14″W, 191 m, 2006, A. Flakus 6570 (LPB); PROV. FEDERICO ROMAN, near Santa Cruzito colony, 10°43′03″S 65°55′05″W, 149 m, 2006, A. Flakus 7107, 7160, 7111, 7257, 7127 (KRAM, LPB, herb. Flakus).

COMMENTS — *Lyromma dolicobelum* is distinguished from all other *Lyromma* species by the few but very long appendages, up to 200–300(–400) µm long and composed of agglutinated hyphae.

Lyromma multisetulatum Flakus & Farkas, **sp. nov.** PLATE 1D; 2A, J

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Differs from *Lyromma pilosum* by its more numerous, larger, and strongly recurved perithecial appendages.

TYPE — Bolivia, Dept. Santa Cruz, Prov. Guarayos, Reserva Vida Silvestre Rios Blanco y Negro, Plan de Manejo AISU, 15°01′58″S 62°46′36″W, 242 m, lowland Amazon forest, 24 July 2009, A. Flakus 13961 & P. Rodriguez (**Holotype**, KRAM; **isotype**, LPB).

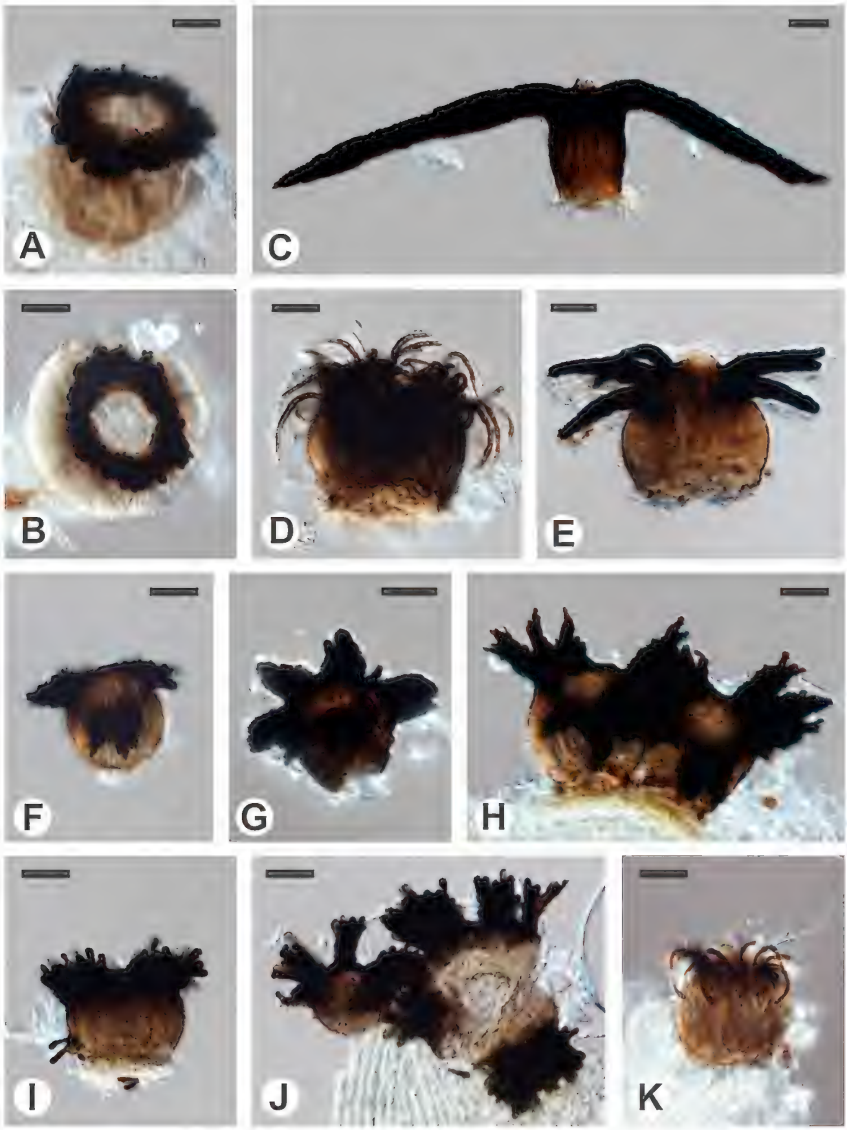

Plate 1. Morphology of perithecial appendages of *Lyromma*. A–B: ***L. coronatum***, reduced, forming a crown around ostiole (holotype). C: ***L. dolicobelum***, tubular, horizontal to recurved, composed of agglutinated hyphae. D: ***L. multisetulatum***, setose, recurved, composed of individual acicular hyphae (holotype). E: ***L. nectandrae***, tubular, horizontal to recurved, composed of agglutinated hyphae. F–H: ***L. ornatum***, triangular to irregularly lobate (H, deeply divided), horizontal, composed of agglutinated hyphae. I–J: ***L. palmae***, setose to brush-shaped, erect, composed of individual clavate hyphae or agglutinated at the base (J, brush-shaped). K: ***L. pilosum***, setose, recurved, composed of individual hyphae. Scale bars = 25 μm.

Etymology — The species is named after its strongly recurved appendages produced in large numbers near the top of perithecia.

Thallus foliicolous, epiphyllous, ecorticate, formed by rounded patches, smooth, 1–2 mm wide, olive-green; photobiont *Phycopeltis*, cells rectangular, 18–22 × 6–8 µm, arranged in radiate plates; perithecia sessile, subglobose to globose, 70–80 µm in diam., and 70–90 µm high, dark brown, around ostiole furnished with (10–)15–30(–40) setae; setae dark brown, septate, strongly recurved, composed of individual hyphae, 30–50(–60) µm long and 3–4 µm broad (2–3 µm broad in apical part); peridium dark brown, lower part slightly paler, 3–4 µm thick, composed of rectangular cells (3–4 µm wide), thick walled (c. 1 µm thick), arrangement irregular to almost parallel; paraphyses sparse in mature perithecia, simple, c. 1 µm thick; asci bitunicate, 8-spored, 30–35 × 10–13 µm; ascospores fusiform, hyaline, 3-septate, with slight constrictions at septa, 14–18 × 4–5 µm; pycnidia elongate, barrel-shaped with tapering top, 100–130 µm high and 80–90 µm wide, dark brown, upper part around ostiole furnished with 15–30 setae; setae dark brown, septate, strongly recurved, composed of individual hyphae, (20–)30–50(–60) µm long and 3–4 µm broad (1–2 µm broad in apical part); conidia (microconidia) bacilliform, non-septate, 2–5 × 0.8–1.2 µm.

Distribution & ecology — The new species is known from the Reserva Vida Silvestre Rios Blanco y Negro, Bolivia and the Private Nature Conservation Reserve founded by Károly Kögl near São Paulo, Brazil. It grows on vascular plant leaves in lowland Amazon forest and Atlantic submontane rainforests.

Additional specimens examined — **BRAZIL. Estado São Paulo.** São Roque, sobre la Lagunita, al Norte de la Laguna Sapucaia, ca 850 m, 1995, A. Borhidi s.n. (VBI).

Comments — *Lyromma multisetulatum* is characterized by its numerous [(10–)15–30(–40)], long [30–50(–60) µm], and strongly recurved perithecial appendages, each composed of an individual hypha. It is the first *Lyromma* species known to produce microconidia. Perithecia of the most similar species, *L. pilosum,* differ in forming less numerous appendages [(5–)10–15] that are less curved and almost twice as short (10–15 µm) (Lücking 2008).

Lyromma nectandrae Bat. & H. Maia, Atas Inst. Micol. Fed. Univ. Pernambuco 2: 360. 1965. Plate 1e

Type — Brazil. Pernambuco: Recife, Dois Irmãos, III 1960, Soares da Silva 13832 (URM 18764 – holotype); Brazil: Amazonas: Manaus, Itacoatiara, VII 1967, Omar 40677 (URM 69375 – epitype, designated by Lücking et al.: 145. 1998).

= *Lyromma confusum* Lücking & Sérus., in Lücking, Fl. Neotrop. Monogr. 103: 187. 2008.

Type — Papua New Guinea, Madang prov. Balek Wildlife Reserve, c. 15 km S of Madang along road to Lae, 5°18′S 145°43′E, c. 20 m, in tropical lowland rainforest, on tree trunk/ on shaded limestone cliff (former coral reef), 29 July 1992, E. Sérusiaux 13555-17 (LG – holotype!).

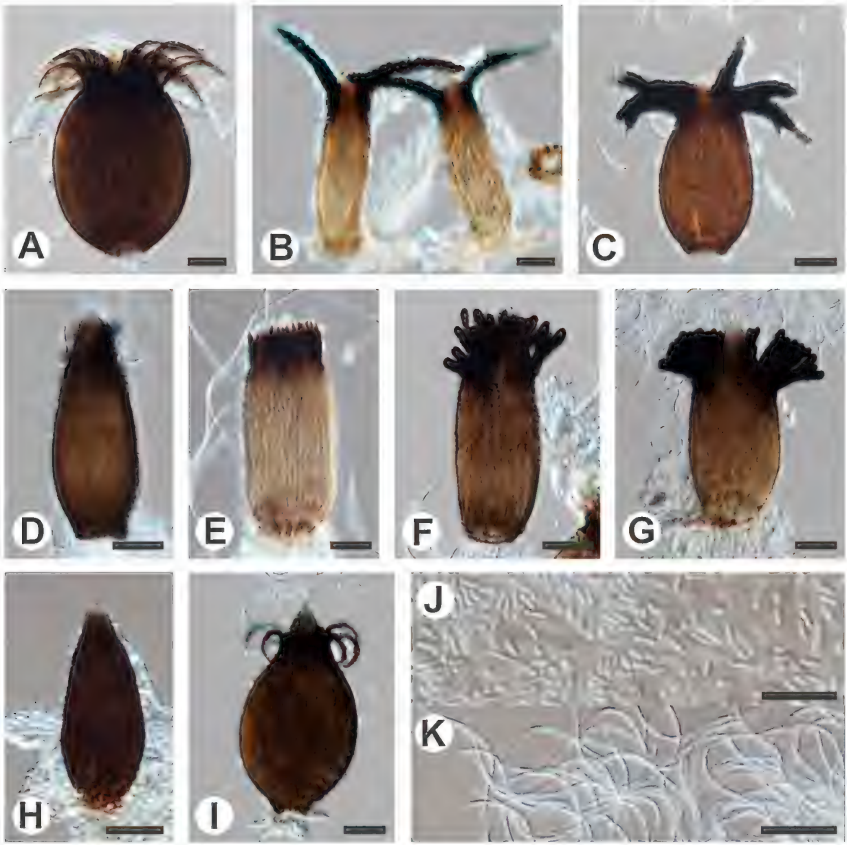

Plate 2. Morphology of pycnidial appendages and anatomy of conidia of *Lyromma*. A: ***L. multisetulatum***, setose, recurved, composed of individual acicular hyphae (holotype). B–C: ***L. ornatum***, tubular (simple to furcate), horizontal to recurved, composed of agglutinated hyphae (B, young pycnidia). D–G: ***L. palmae***, setose, erect, composed of individual clavate hyphae (D, young, glabrous pycnidium). H–I: ***L. pilosum***, setose, recurved, composed of individual acicular hyphae (H, young, glabrous pycnidium). J: ***L. multisetulatum***, short, bacilliform microconidia (holotype). K: ***L. pilosum***, large, filiform macroconidia. Scale bars: A–I, K = 25 μm; J = 10 μm.

"*Lyromma confusum*" Lücking, in Herrera-Campos et al.,
Lichenologist 36: 315. 2004, nom. nud.

[For detailed descriptions see Batista & Maia (1965) and Lücking (2008)].

Distribution & ecology — This pantropical species is rather widespread in the Neotropics, where it is known from Argentina, Brazil, Costa Rica, Ecuador, French Guyana, Mexico, and Panama (Sipman 1997, Ferraro & Lücking 2000, Herrera-Campos et al. 2004, Lücking 2008), and is now also reported from Bolivia, where it was found in lowland rainforests.

Selected specimens examined — **BOLIVIA. Dept. Beni.** Prov. Ballivian, near Pircayo river, 14°18′56″S 67°26′34″W, 190 m, 2004, A. Flakus 4174.1 (LPB). **Dept. Pando.** Prov. Federico Roman, near Santa Cruzito colony, 10°43′03″S 65°55′05″W, 149 m, 2006, A. Flakus 7193.1, 7298 (KRAM, LPB). **Dept. Santa Cruz,** Prov. Guarayos, Reserva Vida Silvestre Rios Blanco y Negro, Virgen de Pilar near Chonta village, 15°38′54″S 62°57′37″W, 229 m, 2009, A. Flakus 13481, 13381.1 & P. Rodriguez (KRAM, LPB); Plan de Manejo AISU, 15°01′58″S 62°46′36″W, 242 m, 2009, A. Flakus 13911 & P. Rodriguez (LPB). **BRAZIL. Estado Tocantins.** Toncinópolis, 3 km al NE de la ciudad, Fazenda de José Borges cerca del Riacho Francisco, 1995, A. Borhidi s.n. (VBI). **Estado Rio de Janeiro.** Serra do Mantiqueira, Parque Nacional de Itatiaia, en el alrededor del Cachueiro Veu da Noiva, ca 1300–1500 m, 1995, A. Borhidi s.n. (VBI). **PAPUA NEW GUINEA. Madang Province.** Pont de la Gogol River, route de Lae à env. 15 km de Madang, 5°20′S 145°43′E, 30 m, 1987, J. Lambinon 87/370 (LG); Ramu valley. Brahman, along road to Bundi, 5°46′S 145°21′E, 200 m, 1992, E. Sérusiaux 14200–2 (LG); Fothills of Finisterre range, along road Madang-Lae, km 39, c. 230 m, 1992, E. Sérusiaux 14303–48 (LG). **Central Province.** N of Brown River, 3 km NE of Hiritano highway, 30–50 m, 1987, J.R. Sloover 87 L 23 (LG). **INDONESIA. Bali.** Gianyar District, Gununk Kawi, 8°28′S 115°18′E, 1991, 500 m, L. Hoffmann 91–115 (LG).

Comments — Although Lücking (2008) considered the perithecial appendages of *Lyromma confusum* morphologically very similar to those on the pycnidia of *L. nectandrae,* he treated the two as separate taxa because there was no proof of a conidial-ascosporic connection. We found thalli bearing pycnidia typical of *L. nectandrae* mixed together with perithecia of *L. confusum* and so regard *L. confusum* as a synonym of *L. nectandrae.*

Mature perithecia of *L. nectandrae* have 3–5, horn-shaped, recurved (rarely horizontal), 30–70 µm long and 10–15(–20) µm broad (at the base) appendages and are sometimes 2–3-furcate at the ends (Lücking 2008). Young undeveloped *L. nectandrae* specimens can be confused with *L. ornatum,* which has short perithecial appendages but which can be distinguished from mature *L. nectandrae* material by the irregularly lobate to triangular, horizontal (rarely slightly recurved), comparatively shorter [15–30(–40) µm long], perithecial appendages (Lücking & Kalb 2000, Lücking 2008).

Likewise, the pycnidia of *L. nectandrae* are morphologically similar to those of *L. ornatum* (shown on Plate 2C) with (2–)3–5 cylindrical to irregular appendages that are 40–60(–100) µm long and 10–15 µm broad (at the base) and may be simple to 2–3-furcate at the ends (Batista & Maia 1965, Lücking 2008).

Lyromma ornatum Lücking, Kalb & Sérus., in Lücking & Kalb, Bot. Jahrb. Syst. 122: 39. 2000. Plate 1F–H; 2B, C

Type — Brasilien. São Paulo, Serra do Mar, Serra do Garrãozinho, zwischen Mogi das Cruzes und Bertioga, in einem primären Regenwald, 23°45′S 46°10′W, 300–600 m, 6.IX.1980, K. Kalb 32601 & G. Plöbst (herb. K. Kalb – holotype!).

[For detailed descriptions of the thallus and perithecia, see Lücking & Kalb (2000) and Lücking (2008)].

Pycnidia elongate barrel-shaped with a tapering top, 100–120 µm high and 35–60 µm in wide, dark brown, upper part around the ostiole furnished with 2–4 setae; setae dark brown to black, recurved, composed of agglutinated hyphae, cylindrical to irregular, simple to 2–4-furcate on the ends, 30–80 µm long and 10–20 µm broad (at the base); conidia (macroconidia) filiform, multiseptate, 50–80 × 0.8–1.2 µm.

Distribution & ecology —This species, rather widespread in the Neotropics, has been reported from Argentina, Brazil, Ecuador, Peru, Mexico, and Surinam (Ferraro & Lücking 2000, Lücking & Kalb 2000, Herrera-Campos et al. 2004, Lücking 2008). These are the first records from Bolivia, where it is known only from lowland Amazon forest.

Selected specimens examined — **BOLIVIA. Dept. Beni.** Prov. Ballivian near Pircayo river, 14°18′56″S 67°26′34″W, 190 m, 2004, A. Flakus 4173 (LPB). **Dept. Pando.** Prov. Manuripi, Reserva Nacional de Vida Silvestre Amazónica Manuripi, Bajada colony near Chive village, 12°24′03″S 68°26′45″W, 170 m, 2006, A. Flakus 6019.1 (LPB); Chive village, 12°23′19″S 68°34′39″W, 157 m, 2006, A. Flakus 6407 (KRAM, LPB); Puerto Rico village, 11°06′12″S 67°31′14″W, 191 m, 2006, A. Flakus 6554, 6565 (KRAM, LPB); Prov. Nicolas Suarez, near Cobija village, 11°02′16″S 68°45′31″W, 191 m, 2006, A. Flakus 6455, 6457 (KRAM, LPB, herb. Flakus); Prov. Madre de Dios, Reserva Nacional de Vida Silvestre Amazónica Manuripi, Puerto Madre de Dios village, 11°30′56″S 67°16′07″W, 153 m, 2006, A. Flakus 6802, 6865 (KRAM, LPB); Prov. Federico Roman, near Santa Cruzito colony, 10°43′03″S 65°55′05″W, 149 m, 2006, A. Flakus 7275 (LPB). **Dept. Santa Cruz.** Prov. Guarayos, Reserva Vida Silvestre Rios Blanco y Negro, Virgen de Pilar near Chonta village, 15°38′54″S 62°57′37″W, 229 m, 2009, A. Flakus 13381, 13382 & P. Rodriguez (KRAM, LPB); Plan de Manejo AISU, 130 km from Ascención de Guarayos village, 15°03′35″S 62°45′20″W, 258 m, 2009, A. Flakus 13841, 13842 & P. Rodriguez (KRAM, LPB, herb. Flakus). **BRAZIL. Estado Tocantins.** Toncinópolis, 3 km al NE de la ciudad, Fazenda de José Borges cerca del Riacho Francisco, 1995, A. Borhidi s.n. (VBI).

Comments — Studies on a wide range of material show that *Lyromma ornatum* exhibits considerable variation in the shape and size of its perithecial appendages. The species forms 3–6 horizontal to (rarely) slightly recurved appendages composed of distinctly agglutinated hyphae, which are triangular to irregularly lobate but sometimes also deeply divided at the apex, 15–30(–40) µm long and 7–15(–20) µm broad (at the base). Morphotypes with strongly incised appendages are externally similar to the perithecia of *L. palmae*. However, *L. palmae* has erect to horizontal appendages, composed of 20–60 clavate hyphae (apically ≤4–7 µm broad), usually grouped into 3–6 brush-shaped appendages (then faintly agglutinated at the base). *Lyromma nectandrae*, another similar species (see above), has much larger recurved perithecial appendages (30–70 µm long) (Lücking 2008).

Only a few poorly developed (probably juvenile) pycnidia were observed on thalli of *L. ornatum* together with perithecia typical for the species. They most closely resemble those of *L. nectandrae* in having recurved simple to

2–4-furcate appendages composed of agglutinated hyphae, but they are less numerous [2–3(–4)] and shorter (30–80 µm long). The relationship between these two species can be clarified only by studying more complete material or by molecular analyses.

Lyromma palmae (Cavalc. & A.A. Silva) Lücking & Sérus., in Lücking et al., Lichenologist 30: 136. 1998. PLATE 11, J; 2D–G

≡ *Anconomyces palmae* Cavalc. & A.A. Silva, in Cavalcante et al., Publ. Inst. Micol. Univ. Fed. Pernambuco 647: 26. 1972.

TYPE — Brazil. Amazonas: Manaus, Manaus, Rodovia AM-1, km 55, Aug 1961, Carvalho 17065 (URM 29109 – holotype; INPA – isotype); Brazil. Maranhão: Zé Doca, IX 1965, Peres 25270 (URM 52420 – epitype, designated by Lücking et al.: 136. 1998).

THALLUS foliicolous, epiphyllous, ecorticate, forming rounded patches, smooth, 0.3–3 mm diam, olive-green; PHOTOBIONT *Phycopeltis*, cells rectangular, 8–21 × 4–7 µm, arranged in radiate plates; PERITHECIA sessile, subglobose to globose, 40–80 µm in diam., and 40–60 µm high, dark brown, around ostiole furnished with 20–60 individual setae or 3–6 brush-shaped appendages; SETAE dark brown, erect to horizontal, composed of about 20–60 individual, clavate hyphae, 10–30(–40) µm long and (3–)4–6(–7) µm broad (at the apex), setose but usually grouped in 3–6 brush-shaped appendages, then faintly agglutinated at the base; PERIDIUM dark brown, lower part slightly paler, 3–4 µm thick, composed of rectangular cells (2–3 µm wide), thick walled (c. 1 µm thick), arrangement irregular to almost parallel; PARAPHYSES sparse in mature perithecia, simple, c. 1 µm thick; ASCI bitunicate, 8-spored, 30–35 × 10–14 µm; ASCOSPORES fusiform, hyaline, 3-septate, with slight constrictions at septa, second cell from above sometimes slightly enlarged, 14–18 × 3–5 µm; PYCNIDIA elongate barrel-shaped with a tapering top, 100–130 µm tall and 40–55 µm wide, dark brown, upper part around the ostiole furnished with 20–25 setae; SETAE dark brown, erect, usually clavate, septate, composed of individual hyphae, (10–)20–30(–40) µm long, 3–4 µm broad in basal part and (3–)4–6(–7) µm broad in apical part; CONIDIA (macroconidia) filiform, multiseptate, 40–100 × 0.8–1.2 µm.

DISTRIBUTION & ECOLOGY — This species is known from Argentina, Bolivia, Brazil, Costa Rica, and Mexico (Cavalcante et al. 1972, Lücking et al. 1998, Ferraro & Lücking 2000, Herrera-Campos et al. 2004, Flakus & Lücking 2008, Lücking 2008). It is found mostly in lowland rainforests, and also in northern Argentinean forests.

SELECTED SPECIMENS EXAMINED — **BOLIVIA. DEPT. BENI.** PROV. BALLIVIAN, Nuevos Reyes colony, 14°13′17″S 67°28′25″W, 190 m, 2004, A. Flakus 3811 (LPB); near Pircayo river, 14°18′56″S 67°26′34″W, 190 m, 2004, A. Flakus 4174.1, 4195.1 (KRAM, LPB). **DEPT. PANDO.** PROV. MANURIPI, Reserva Nacional de Vida Silvestre Amazónica Manuripi, Bajada colony near Chive village, 12°24′03″S 68°26′45″W, 170 m, 2006, A.

Flakus 5989.3 (KRAM); Puerto Madre de Dios village, 11°27′38″S 67°15′56″W, 166 m, 2006, A. Flakus 6662, 7046 (KRAM, LPB). **Dept. Santa Cruz.** Prov. Guarayos, Reserva Vida Silvestre Ríos Blanco y Negro, Virgen de Pilar near Chonta village, 15°38′54″S 62°57′37″W, 229 m, 2009, A. Flakus 13332, 13491 & P. Rodriguez (KRAM, LPB); Plan de Manejo AISU, 130 km from Ascención de Guarayos village, 15°03′35″S 62°45′20″W, 258 m, 2009, A. Flakus 13842.1, 13842.2 & P. Rodriguez (KRAM, LPB); 160 km from Ascención de Guarayos village near Río Negro, 14°58′50″S 62°36′19″W, 242 m, 2009, A. Flakus 14021 & P. Rodriguez (herb. Flakus).

Comments — The perithecia of *L. palmae* are characterized by the erect to horizontal setae composed of 20–60 clavate hyphae grouped usually into 3–6 brush-shaped appendages, 10–30(–40) µm long and (3–)4–7 µm broad at the apex. This distinguishes it from the most similar species, i.e. *L. coronatum*, *L. ornatum* (see also below), and *L. pilosum*.

The structure of the pycnidia and pycnidial appendages of *L. palmae* were variously interpreted before 2008. According to Lücking et al. (1998), the species was characterized by pycnidia with reduced apical setae, compared to those of, for example, *L. nectandrae* or *L. dolicobelum*. In his subsequent monograph, Lücking (2008) described *L. palmae* as having glabrous pycnidia without appendages. However, the original description and illustration (Cavalcante et al. 1972: 26, fig. 5), as well as a detailed study of the epitype by Lücking et al. (1998: 136) established that the species develops small appendages near the pycnidial apex. The rich collections now available show that *L. palmae* is characterized by pycnidia with 20–25 erect usually clavate setae composed of individual hyphae that are (10–)20–30(–40) µm long, 3–4 µm broad at the base, and (3–)4–6(–7) µm broad at the apex (Plate 2 F, G). Pycnidia of *L. palmae* are somewhat reminiscent of those of *L. pilosum* but differ in having acicular, horn-shaped, and strongly recurved appendages (1–3 µm broad at the apex).

Lyromma pilosum Lücking, Fl. Neotrop. Monogr. 103: 187. 2008. Plate 1K; 2H, I, K

Type — Costa Rica. Puntarenas: Monteverde Biological Reserve, 1600–1700 m, Feb 2000, Lücking 00-418 (CR – holotype).

[For a detailed description of the thallus and perithecia, see Lücking (2008).]

Pycnidia elongate barrel-shaped with tapering top, 100–130 µm tall and 40–60 µm in wide, dark brown, upper part around ostiole furnished with 5–10 setae; setae dark brown, acicular, horn-shaped, strongly recurved, septate, composed of individual hyphae, (10–)20–30(–40) µm long, and 4–5 µm broad (2–3 µm broad in apical part); conidia (macroconidia) filiform, multiseptate, 40–70 × 0.8–1.2 µm.

Distribution & ecology — This species was described from Argentina and Costa Rica (Lücking 2008). Here we report it from Bolivia and Brazil for the first time. It grows in northern Argentinean forests, montane forest, lowland Amazon forest, and Atlantic submontane rainforest.

Selected specimens examined — **BOLIVIA. Dept. Pando.** Prov. Manuripi, Reserva Nacional de Vida Silvestre Amazónica Manuripi, Chive village, 12°23′47″S 68°35′28″W, 179 m, 2006, A. Flakus 5900, 5903.2 (KRAM, LPB); Bajada colony near Chive village by Río Madre de Dios, 12°24′03″S 68°26′45″W, 170 m, 2006, A. Flakus 5975, 6019.2 (LPB, herb. Flakus); Prov. Nicolas Suarez, near Cobija village, 11°02′16″S 68°45′31″W, 191 m, 2006, A. Flakus 6440 (LPB); Prov. Madre de Dios, Reserva Nacional de Vida Silvestre Amazónica Manuripi, near Puerto Madre de Dios village, near Río Madre de Manupare, 11°31′37″S 67°17′29″W, 155 m, 2006, A. Flakus 6719 (KRAM). **BRAZIL. Estado São Paulo.** São Roque, cascada sobre la Laguna Sapucaia, ca 800 m 1995, A. Borhidi s.n. (VBI); sobre la Laguna Sapucaia, arriba del camino, ca 850 m, 1995, A. Borhidi s.n. (VBI).

Comments — *Lyromma pilosum* was the first species of the genus described as having short perithecial appendages composed of an individual hypha (Lücking 2008). It is most similar to *L. multisetulatum* and *L. palmae*, which differ in the size, shape, and orientation of the perithecial appendages. For more details, see remarks below those species.

The pycnidia of *L. pilosum* and *L. palmae* have similar appendages, each composed of individual hypha that is 3–5 µm broad across the base. Juvenile pycnidia of both species are smooth (without appendages) and can be distinguished only in the mature stage. Pycnidia of *L. pilosum* have 5–10 setae, which are acicular, strongly recurved, (10–)20–30(–40) µm long, and 2–3 µm broad at the apex. In contrast, those of *L. palmae* produce 20–25 setae, which are erect, usually clavate, (10–)20–30(–40) µm long, and (3–)4–6(–7) µm broad at the apex.

Key to species of *Lyromma* based on ascosporic and conidial states

1a. Sporocarps perithecia, globose to very short barrel-shaped, usually <100 µm high . . .2
1b. Sporocarps pycnidia, elongate barrel-shaped, usually >100 µm high 8

2a (1a). Perithecial appendages (5–)10–30(–60), setose (strongly recurved) to brush-shaped (erect to horizontal), each composed of individual hypha (sometimes faintly agglutinated at the base), (5–)10–30(–40) µm long and 3–5 µm broad at the base) or almost reduced, forming a crown 3
2b. Perithecial appendages 2–6, horn-shaped or triangular to irregularly lobate, horizontal to recurved, composed of agglutinated hyphae (sometimes deeply divided at the apex), 15–200(–300) µm long and 7–30 µm broad (at the base) 6

3a (2a). Perithecial appendages reduced, forming a crown, composed of individual clavate hypha, 5–7(–12) µm long and (4–)5–7 µm broad (at the apex) . . . . . ***L. coronatum***
3b. Perithecial appendages larger, strongly recurved, setose 10–50(–60) µm long or erect to horizontal, composed of 20–60 individual hyphae usually grouped into 3–6 brush-shaped appendages, 10–30(–40) µm long 4

4a (3b). Perithecial appendages erect to horizontal, composed of 20–60 clavate hyphae usually grouped into 3–6 brush-shaped appendages (then faintly agglutinated at the bases), 10–30(–40) µm long and (3–)4–7 µm broad (at the apex) ..... ***L. palmae***

4b. Perithecial appendages (5–)10–30(–40) strongly recurved setae composed of acicular hyphae, 10–50(–60) µm long and 2–3 µm broad (at the apex) 5

5a (4b). Perithecial appendages (5–)10–15 setae, 10–30(–40) µm long ..... ***L. pilosum***

5b. Perithecial appendages (10–)15–30(–40) setae, 30–50(–60) µm long ..... ***L. multisetulatum***

6a (2b). Perithecial appendages 3–6, triangular to irregularly lobate (sometimes deeply multi-furcated on the apex), horizontal to rarely slightly recurved, 15–30(–40) µm long and 7–15(–20) µm broad (at the base) ..... ***L. ornatum***

6b. Perithecial appendages 2–5, cylindrical to irregular, horn-shaped (sometimes 2–3-furcate on the ends), recurved or rarely almost horizontal, 30–200 µm long and 10–25 µm broad (at the base) 7

7a (6b). Perithecial appendages 3–5; 30–70 µm long, simple to 2–3-furcate on the apex ..... ***L. nectandrae***

7b. Perithecial appendages 2–3(–4); 60–200(–300) µm long ..... ***L. dolicobelum***

8a (1b). Pycnidia glabrous (when young) or with 5–30 appendages composed of individual hyphae, 3–5 µm broad (at the base) 9

8b. Pycnidia with 2–5 appendages composed of agglutinated hyphae, 10–20 µm broad (at the base) 11

9a (8a). Appendages 20–25, clavate, erect to almost vertical, (10–)20–30(–40) µm long and (3–)4–6(–7) µm broad (at the apex) ..... ***L. palmae***

9b. Appendages acicular, horn-shaped, strongly recurved, 1–3 µm broad (at the apex) 10

10a (9b). Appendages 5–10, (10–)20–30(–40) µm long; macroconidia filiform, multiseptate, 40–60 × 0.8–1.2 µm ..... ***L. pilosum***

10b. Appendages 15–30, (20–)30–50(–60) µm long; microconidia bacilliform, non-septate, 2–5 × 0.8–1.2 µm ..... ***L. multisetulatum***

11a (8b). Appendages (2–)3–5, simple to 2–4-furcate on the ends, cylindrical to irregular, 30–60(–100) µm long and 10–20 µm broad (at the base) ..... ***L. nectandrae*** or ***L. ornatum***

11b. Appendages 2(–3), simple, cylindrical, 200–400 µm long and 10–20 µm broad (at the base) ..... ***L. dolicobelum***

Discussion

During the current study, we discovered undescribed ascosporic states for *Lyromma dolicobelum* and *L. palmae* and conidial states for *L. ornatum* and *L. pilosum*. For the first time in these species both perithecia and pycnidia were observed associated on the same thallus under natural conditions. A conidial-

ascosporic connection for *Lyromma nectandrae* suggests that *L. confusum* should be treated as a synonym of that species. Additionally, two new species of the genus, *Lyromma coronatum* and *L. multisetulatum*, were discovered in material from Bolivia and Brazil.

Since all *Lyromma* taxa have very similar thalli, their relationships have been based on the morphology and anatomy of their sporocarps, with the most useful characters provided by the variable appendages produced near the ostiole both in the ascosporic and the conidial states. From our taxonomic revision of additional, recently collected material, we propose a new concept of the conidial-ascosporic relationships in this foliicolous genus. Despite the many differences between perithecia and pycnidia noted by Lücking (2008), our results show similarities between appendage types that connect ascosporic and conidial states for each species.

Our hypothesis on the generic circumscription, which is probably temporary due to the still limited knowledge on this overlooked genus, can be expected to evolve as new collections and/or molecular sequence data becomes available. The size of these organisms (with a mean thallus diameter normally <5 mm, and sporulating structures around 100 μm high) also limits further studies. We hope, however, that our revised key to *Lyromma* species using both perithecial and pycnidial characters will encourage further research into this fascinating foliicolous lichenized genus.

Acknowledgments

We are very grateful to Prof. David Hawksworth (London), Dr. Robert Lücking (Chicago), and Dr. Shaun Pennycook (Auckland) for reviewing the manuscript, and for valuable and constructive comments on previous versions of this paper. Mr. Ken Hudson (Egham) and Dr. David Minter (Egham) are thanked for clarifying bibliographic problems concerning to cited publications. We are also greatly indebted Prof. Klaus Kalb (Neumarkt) and Prof. Emmanuël Sérusiaux (Liège) for loan of the type material for study, Dr. Martin Kukwa (Gdańsk) for valuable comments, as well as to Rosa I. Meneses Q., the Director of Herbario Nacional de Bolivia, Universidad Mayor de San Andrés, La Paz for generous cooperation. This research received support from the NCN in Poland for the years 2008–2011 (no. N N303 345335), the NCBiR in Poland under the LIDER Programme for the years 2010–2013 (no. 92/L–1/09), the W. Szafer Institute of Botany of the Polish Academy of Sciences through the program supporting research of young scientists, and the Hungarian Scientific Research Fund (OTKA K81232).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.143

Volume 124, pp. 143–148 *April–June 2013*

Scolecobeltrania, an interesting new microfungus from Venezuela

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Abstract — A new microfungus collected on dead plant material from the "Cerro El Ávila" mountain range and "Parque Nacional Canaima" tropical forest in Venezuela is described and illustrated. *Scolecobeltrania vermispora* gen. & sp. nov. is characterized by macronematous branched conidiophores, polyblastic conidiogenous cells, and unicellular vermiform to sigmoid pale brown conidia with up to 11 hyaline transverse bands.

Key words — anamorphic fungi, systematics, leaf litter

Introduction

During two mycological surveys of anamorphic fungi in the cloud and tropical forests in Venezuela, we collected an interesting conspicuous fungus. It has some characters that are present in *Beltrania* and some allied genera, but the conidia differ morphologically from those in all previously described anamorphic genera of this group. We propose a new genus to accommodate this new fungus described here.

Plant samples were placed in paper and plastic bags, taken to the laboratory, and treated according to Castañeda (2005). Mounts of fungal material were prepared in polyvinyl alcohol-glycerol (8 g in 100 ml of water, plus 5 ml of glycerol) and diagnostic characters were measured at ×1000. Micrographs were obtained with a Zeiss Axioskop 40.

Taxonomy

Scolecobeltrania Iturr., R.F. Castañeda & Rob. Fernández, **gen. nov.**

MycoBank MB801469

Differs from *Beltraniella* by polyfasciate scolecoconidia and from *Pseudobeltrania* by setae intermixed with conidiophores and polyfasciate scolecoconidia.

Type Species: *Scolecobeltrania vermispora* Iturr. et al.

Etymology: from *Scoleco-*, referring to conidia with a length/width radio >15:1 + *beltrania*, name of a similar anamorphic fungal genus.

Colonies on the natural substratum amphigenous, effuse, coalescent, felted to compact floccose, forming conspicuous patches, brown to olivaceous or black. Mycelium superficial and immersed. Setae erect, straight or sinuate, septate with or without radially lobed basal cell, smooth or verruculose, brown. Conidiophores macronematous, mononematous, erect, branched, septate, brown to olivaceous, smooth or verruculose. Conidiogenous cells monoblastic and polyblastic, discrete, determinate or indeterminate, with holoblastic sympodial proliferations. Conidial secession schizolytic. Conidia scolecosporous, solitary, unicellular, sigmoid, vermiform to long cylindrical, curved or sinuate, brown to olivaceous, always with several hyaline or subhyaline transverse bands, smooth or verruculose. Teleomorph unknown.

Scolecobeltrania vermispora Iturr., R.F. Castañeda & Rob. Fernández, **sp. nov.**

Figs. 1 –2

MycoBank MB801470

Differs from *Beltraniella* spp. in its polyfasciate scolecoconidia and from *Pseudobeltrania* spp. in its multiseptate setae intermixed with conidiophores and multifasciate scolecoconidia.

Type: Venezuela, Bolivar State, tropical forest, Luepa, Sector Gran Sabana, Parque Nacional Canaima, 5°50′N 61°27′W, 880–1250 m alt., on decaying leaves of unidentified plant, 25.IV.2005, coll. Teresa Iturriaga (**Holotype**: VEN108367).

Etymology: Latin, *vermispora*, referring to worm-shaped conidia.

Colonies on the natural substrate, effuse, felted forming extensive parches, often coalescence, olivaceous-brown. Mycelium mostly superficial and immersed. Hyphae septate, 2–4 µm diam, profusely branched, densely anastomoses, sometimes forming a rudimentary stroma with somewhat similar "textura intricata", smooth, brown to pale brown. Setae erect, straight or curved to sinuate, cylindrical, acerose or lanceolate at the apex, 7–12-septate, 150–250 µm tall, 10–15 µm diam. at the radially lobed base, smooth, dark brown below, brown towards the apex. Conidiophores macronematous, mononematous, erect, dichotomous, trichotomous or irregularly branched, forming more or less a cluster at the high level, arising from superficial hyphae, 20–35 × 10–12 µm, smooth, pale brown to pale olivaceous-brown. Conidiogenous cells

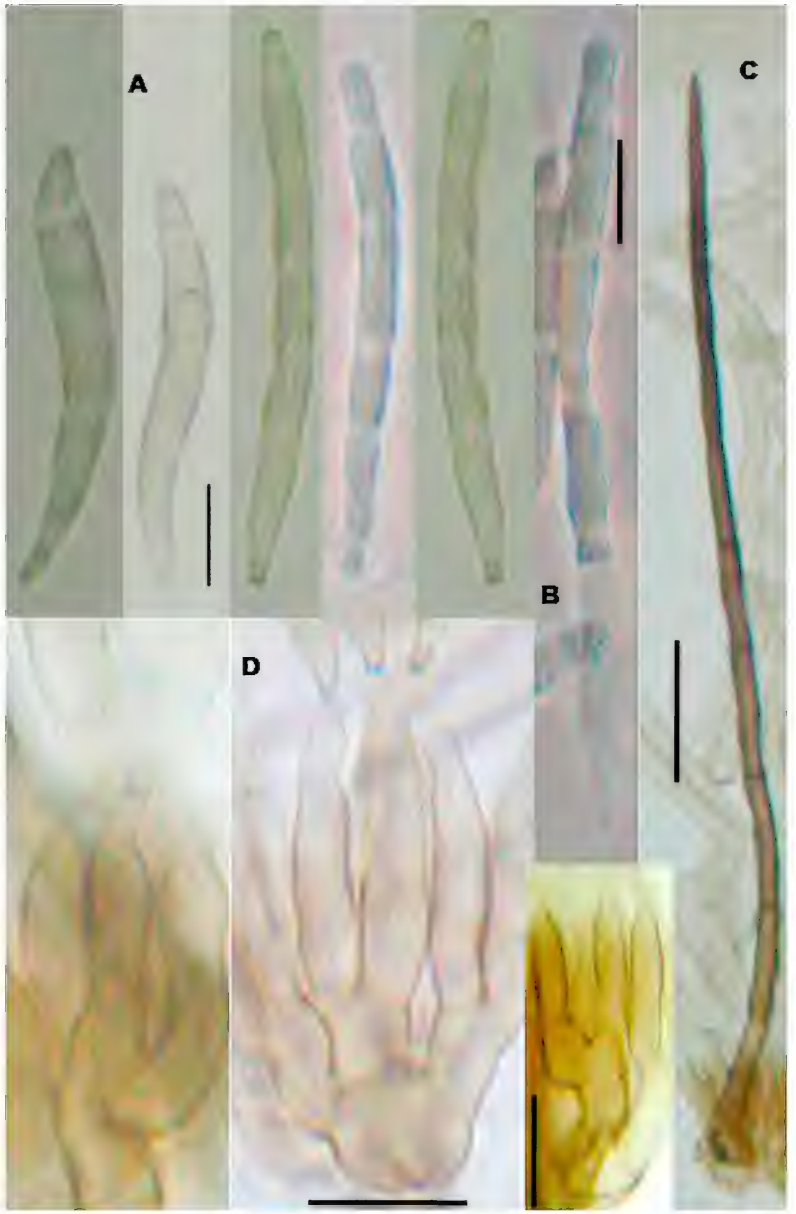

Fig. 1. *Scolecobeltrania vermispora* (ex holotype VEN108367).
A–B. Conidia. C. Seta. D. Conidiophores and conidiogenous cells.
Scale bars: A–B, D = 10 µm, C=25µm.

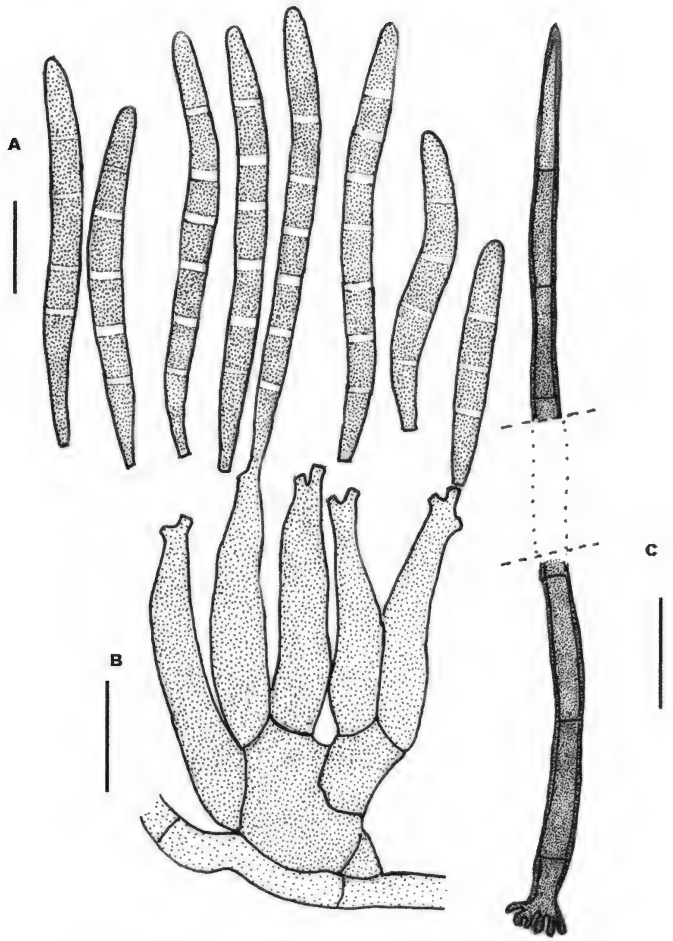

Fig. 2. *Scolecobeltrania vermispora* (ex holotype VEN108367). A. Conidia. B. Conidiophore and conidiogenous cells. C. Seta. Scale bars: A–B = 10 µm, C= 25 µm.

polyblastic, discrete, cylindrical, but slightly attenuate near the apex, denticulate with conspicuous denticles, somewhat opaque at the conidiogenous loci, indeterminate, with several sympodial proliferations, 8–12 × 3–3.5 µm, pale brown. Conidia solitary, vermiform, sigmoid, attenuate and truncate at the base, obtuse to rounded at the apex unicellular, 40–65 ×5.5–8.5 µm, smooth, dry, fasciate, pale brown to pale olivaceous brown, with (2–)5–11 transverse hyaline bands across the length of conidial body. Teleomorph unknown.

Additional specimen examined: **VENEZUELA, Caracas**, "Cerro El Ávila", Parque Nacional El Ävila, 10°33′N 66°51′W, on decaying leaves of *Clusia hilariana* Schltdl., 20.II.2008, coll. R Fernández INIFAT 2461.

Note: Seifert et al (2011) have provided a key, descriptions, and illustrations of *Beltrania* and similar anamorphic genera. Among these fungi the conidiophores and conidiogenous cells of *Beltraniella* Subram. and *Pseudobeltrania* Henn. are morphologically similar to *Scolecobeltrania*. Several *Beltraniella* species have irregular branched conidiophores with branches arising near the bases of straight thick-walled dark brown setae; similar conidiophores also occur in *Pseudobeltrania*, but it lacks setae. The conidia in *Beltraniella* are unicellular, turbinate, and oblageniform (or nearly so) with a hyaline transverse band frequently arising from separating cells or from conidiogenous loci (Pirozynski 1963, Castañeda Ruiz et al. 1996, Kendrick 2003, Seifert et al. 2011), and *Beltraniella* species with supra-equatorial subhyaline or hyaline (pore-like) spots have recently been segregated into the genus *Porobeltraniella* (Gusmão 2004). *Pseudobeltrania* has unicellular biconical olivaceous brown conidia with a transverse hyaline band near the widest part arising directly from conidiogenous loci (Heredia et al. 2002). Scolecoconidia with several transverse hyaline bands originating directly from conidiogenous loci are conspicuous unique characters that clearly separate *Scolecobeltrania* from the other genera.

Acknowledgments

The authors express their sincere gratitude to Dr. Xiu-Guo Zhang and Dr. De-Wei Li for their critical review of the manuscript. The authors are very grateful to Fondo Nacional de Ciencia, Tecnologia e Innovacion (Fonacit, Venezuela) for funding Project .N° S1-2001000663 "Sistemática Molecular y Morfológica de Hongos Ascomycetes y Basidiomycetes Presentes en las Sucesiones Fúngicas Descomponedoras de Madera en Bosques Tropicales"; to the Cuban Ministry of Agriculture for facilities. Collecting in Venezuela has been possible through permits obtained from INPARQUES-0721 and MARNR-1-096, institutions to whom we are indebted. The authors thank Drs Uwe Braun, Pedro Crous, De-Wei Li, and Gregorio Delgado for their generous and valued assistance with literature not otherwise available. We thank Beatriz Ramos and Aliana Sosa for technical assistance. We also acknowledge the facility provided by Dr P.M. Kirk and Drs V. Robert and G. Stegehuis through the Index Fungorum and Mycobank websites. Dr Lorelei L. Norvell's editorial review and Dr Shaun Pennycook's nomenclature review are greatly appreciated.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.149

Volume 124, pp. 149–154 *April–June 2013*

A new species of *Pisolithus* from Spain

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Abstract — *Pisolithus calongei* is described as a new species based on our morphological observations and comparison of ITS nrDNA sequences.

Key words — *Basidiomycota*, *Cistus*, *Boletales*, taxonomy, *Pisolithus tinctorius*

Introduction

During their overview of the donkey dung fungus (*Pisolithus* Alb. & Schwein.) from many regions of the world using molecular and classical methods, Phosri et al. (2012) analysed collections from Europe. All generated molecular trees grouped Spanish specimens into three clades, of which two represent the classic *Pisolithus tinctorius* (Pers.) Coker & Couch and *P. arhizus* (Scop.) Rauschert. However, four sequences obtained from *Pisolithus* specimens associated with *Cistus ladanifer* L. (*Cistaceae*) formed a separate group more closely related to *P. marmoratus* (Berk.) E. Fisch. (occurring in *Eucalyptus* spp. plantations from Australia, China, and Kenya) than to other Spanish specimens.

Cistaceae is well documented as an ectomycorrhizal phanerogam family (Harley & Harley 1987), although many species also form VA and ectendomycorrhizas. Association-forming species are widespread amongst the larger fungi, e.g. *Leccinum arenicola* Watling & Redhead, found in North America with *Hudsonia* L. (Redhead & Watling 1979), and *Terfezia alsheikhii* Kovács et al., found in Spain with *Helianthemum* Mill. (Kovács et al. 2011). In Europe a number of fungi associate with *Helianthemum*, including *Boletus* spp. (Águeda et al. 2006, 2008; Ponce et al. 2011), *Laccaria laccata* (Scop.) Cooke (Torres et al. 1995), and *Cortinarius* spp. and *Hebeloma* spp. (Watling 1988; Moreau 2002; Henrici 2003, 2005). Even *Astraeus hygrometricus* (Pers.)

Morgan, a close relative of *Pisolithus,* has been associated with *Cistus* in Spain (Martín 1988, specimens in BCN; M. Jeppson personal collection with duplicate specimens in E).

Recently, three *Pisolithus* specimens were collected near *Cistus ladanifer* in Spain. Phylogenetic analyses performed according to Phosri et al. (2012) separated the specimens into two distinctive taxa (Fig. 1): one collection (MA-Fungi 47734 (duplicates at BH & E): J.C. Campos & E. Ramírez MPM 3320 collectors near El Robledillo, 435-465 m alt. on 4-Dec-2010; GenBank: HE578142) grouped in the *'tinctorius'* clade and the other two collections (MA-Fungi 47732, MA-Fungi 47733) grouped in the "*Pisolithus* species 3" subclade from Spain together with sequences AF228641–AF228644 (Díez et al. 2001) from specimens from Alcalá de Henares Univ. Herbarium (AH) and FR748140/ FR748142 (Phosri et al. 2012) from the MJ6192 collection (M. Jeppson personal herbarium) revised here.

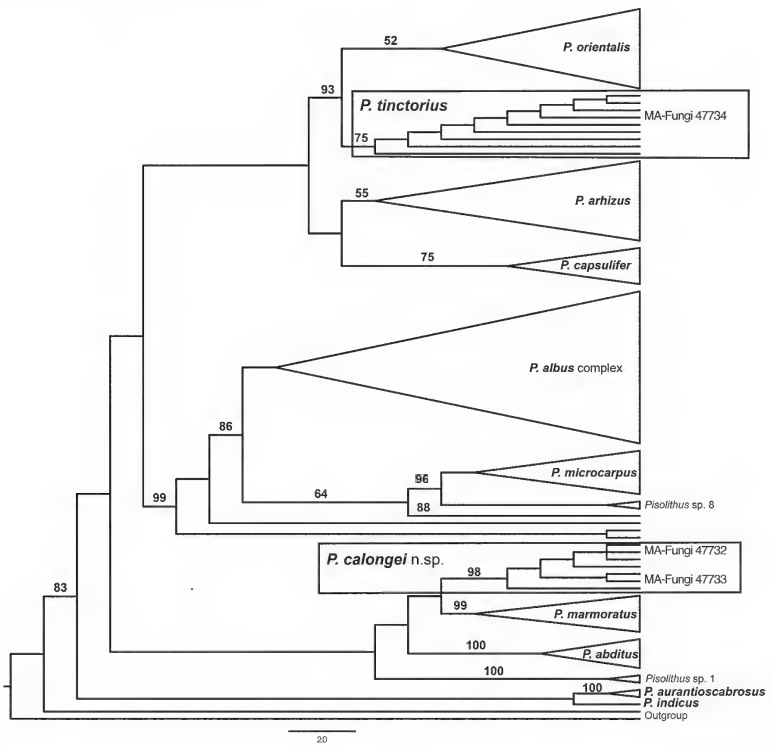

Fig. 1. *Pisolithus calongei* and other *Pisolithus* spp. One of the 100 most parsimony trees obtained under heuristic search (PAUP* 4.0b10) using the sequences included in Phosri et al. (2012) and three newly generated sequences from our *Pisolithus* collections from Spain. The parsimony bootstrap values (%) are indicated on the branches. Bar = 20 changes.

Identification of microscopic features distinctive to MA-Fungi 47732 and MA-Fungi 47733 associated with *C. ladanifer* further support our belief that the specimens represent a new species, which we describe here.

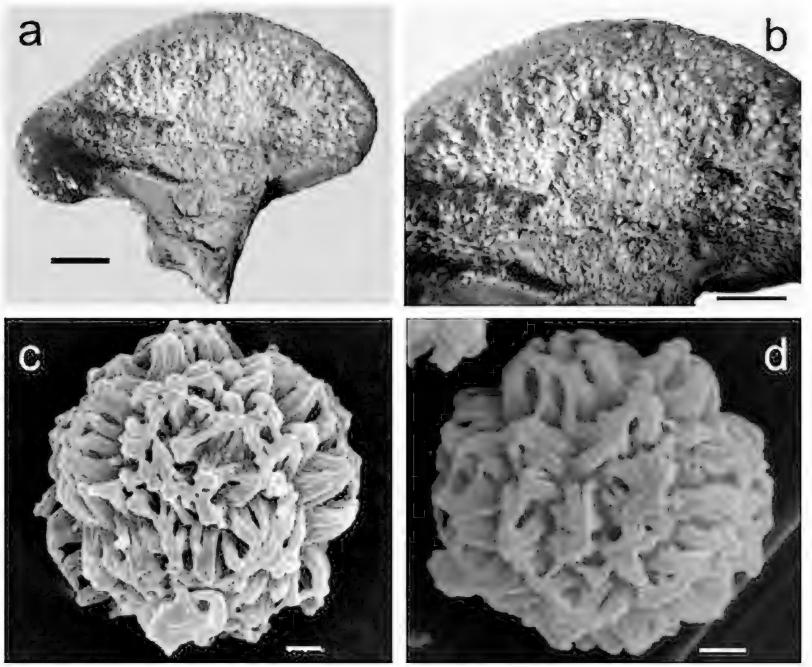

Fig. 2. *Pisolithus calongei.* a–b (MA-Fungi 47732): Turbinate gastrocarp. c–d (Holotype, MA-Fungi 47733): Basidiospores (SEM) coarsely ornamented by broad connate structures that form irregular pyramid-like spines. Scale bars: a = 1 cm; b = 0.5 cm; c–d = 1 µm.

Taxonomy

Pisolithus calongei M.P. Martín, Phosri & Watling, **sp. nov.** Figs 2–3

MycoBank MB 563437

Differs from *Pisolithus tinctorius* and *P. arhizus* by its turbinate habit and from all *Pisolithus* spp. in its ITS nrDNA sequence.

Type: Spain. Ávila: Candeleda, Las Atalayas, 30TU-00477, 355 m alt., close to *Cistus ladanifer*, 11-Dec-2010, leg. E. Ramírez & J. C. Campos MPM 3319 (**Holotype**, MA-Fungi 47733; **isotypes**, BH, E; GenBank, HE578141).

Etymology: Honouring Francisco D. Calonge, who has worked so diligently on the Iberian gasteromycete flora.

Gastrocarp turbinate, pulvinate, 32–40 mm broad, 28–45 mm high, with shortened stipe 6–14 × 10–13 mm, with abrupt base, ± attached to soil mass, main primary peridial layer 15–24 mm deep. Peridium irregular, at most 0.5

Fig. 3. *Pisolithus calongei* (M. Jeppson MJ6192). Turbinate gastrocarp.

mm thick, but soon thinning to show outline of peridioles below, even stretching to accommodate complete peridioles, finally disintegrating and leaving small islands of original ferruginous peridium above yellow-ochre peridiole surfaces or bruising to form lacquer-like blackened smudges. Peridioles 1–1.5 mm diam. (rarely <2 mm), ochraceous yellow at first, later ferruginous brown, compressed in uppermost part, often forming in stipe apex or even developing within the entire stipe to produce irregular rugulose outer surface on the stipe, concolorous with and non-differentiated from the peridial surface; upper peridium finally eroding away to expose the disintegrating peridioles as a dry ferruginous powdery mass. Basidiospores globose 8.6–11 µm diam., coarsely ornamented with broadly connate structures that form irregular pyramid-like spines, 1.5 µm high, rarely collapsing to give broader structures.

Additional specimens examined: **SPAIN. Ávila**: Candeleda, Las Atalayas, 30TU-00477, 350 m alt., close to *Cistus ladanifer*, 4-Dec-2010, leg. E. Ramírez & J. C. Campos MPM3318 (MA-Fungi 47732; duplicate, BH; GenBank, HE578140). **Huelva**: Las Palmas, 1-Jan-2003, leg. M. Jeppson MJ6192 (M. Jeppson personal herbarium).

Pisolithus calongei is distinctive in the field in its turbinate habit, close fruiting to the soil-surface and therefore being concealed by the *Cistus* with which it is thought to associate. The collectors observed no *Pinus* species in the vicinity. The new species may have been overlooked in the past or identified as *P. arhizus* or *P. tinctorius*. Nonetheless, the ITS nrDNA sequence analysis (Fig. 1) clearly separates *P. calongei* from the other three European species

(*P. arhizus*, *P. capsulifer* (Sowerby) Watling et al., and *P. tinctorius*), as well as from the other species previously analyzed: *P. albus* complex, *P. abditus* Kanch. et al., *P. aurantioscabrosus* Watling, *P. indicus* Natarajan & Senthil., *P. marmoratus* (Berk.) E. Fisch., *P. microcarpus* (Cooke & Massee) G. Cunn., and *P. orientalis* Watling et al. (Watling et al. 1995, Martin et al. 2002, Kanchanaprayudh et al. 2003a,b, Moyersoen et al. 2003, Reddy et al. 2005, Phosri et al. 2012).

Acknowledgments

We thank M. Jeppson (Sweden) and A.J.S. Whalley (John Moores University, Liverpool) for presubmission review. Thanks to E. Ramírez, J.C. Campos, and J.C. Zamora for providing the specimens included in this study, to J. Jeppson for permission to reproduce Fig. 3 (specimen M. Jeppson MJ6192), and to Y. Ruiz for her technical assistance with the SEM pictures of the holotype. This work was supported in part by project REN2001-1842/GLO.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.155

Volume 124, pp. 155–163 *April–June 2013*

A reassessment of excavated *Tuber* species from China based on morphology and ITS rDNA sequence data

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Abstract — A composite study was made of the morphology and phylogeny of Chinese *Tuber* species that produce ascomata with a cavity at the base (excavated ascomata). One new species, *T. neoexcavatum,* was found and is here described. It is characterized by brown ascomata with distinctly verrucose surface and prosenchymatous peridium. The presence of *T. excavatum* in China is still in doubt. A key is provided for all excavated species of *Tuber.*

Key words — *Ascomycota*, molecular analysis, taxonomy, truffle

Introduction

Species of *Tuber* that produce distinct cavities at the base of ascomata are often referred to as 'excavated species.' The ascomal surface within the cavity of such species differs considerably from the surface covering the outer ascomata (Cao 2010). For example, *T. pseudohimalayense* (= *T. pseudoexcavatum*) produces larger pyramidal warts inside the cavities but smaller ones outside the cavities, even forming a distinct area with the same large pyramidal warts in some abnormal ascomata that lack cavities. The only exception is *T. mesentericum* Vittad., in which pyramidal warts completely cover the ascomal surface (Riousset et al. 2001).

Occasionally other *Tuber* species (e.g., *T. nitidum* Vittad. and *T. taiyuanense* B. Liu in the Rufum group; *T. liyuanum* L. Fan & J.Z. Cao in the Puberulum group) produce an umbilicus or a distinct pit at the base of an ascoma, giving them the appearance of an excavated species. However, these *Tuber* species could never be mistaken for excavated species, because there is no clear difference between the cavity interior and exterior inside and a cavity is not consistently present within all ascomata in the collection.

Tuber species with true cavities were first described by Vittadini in 1831 (*T. excavatum* and *T. mesentericum*) and Quélet in 1880 (*T. fulgens*). Two Chinese species — *T. pseudoexcavatum* (Wang et al. 1998) and *T. sinoexcavatum* (Fan et al. 2011) — also produce ascomata with cavities. During intensive investigation of the Chinese *Tuber* species with excavated ascomata, the authors found a new excavated species. Here we describe the new species, discuss all three Chinese excavated species, and reassess their taxonomic status.

Materials & methods

Morphological studies

Fresh specimens of the new species described here were collected from Baoshan City, Yunnan Province, P.R. China and deposited in BJTC (Herbarium Biology Department, Capital Normal University in Beijing). The dried specimens were deposited in BJTC and HKAS (Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences). Macro- and microscopic characters were described from fresh specimens and rehydrated specimens. Sections were made with a razor blade and mounted in 3% KOH for observation. Specimens were dried for archival purposes, and permanent slides were prepared by staining in Melzer's reagent, rinsing in water and mounting in polyvinyl lactic glycerol. For scanning electron microscopy (SEM), ascospores were scraped from fresh or dried gleba onto doubled–sided tape mounted directly on an SEM stub, coated with gold–palladium, and examined and photographed with a Hitachi S–4800 SEM.

Molecular methods

Samples from herbarium material were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLabE.Z.N.A._Fungal DNA kit following the manufacturer's protocol. The ITS regions were amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 µl reactions containing DNA template 2 µl, primer (10 µM) 2 µl each, and 2 × Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 µl. PCR reactions were run as follows: an initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 2 min, 55 °C for 25 s, 72 °C for 2 min and a final extension at 72 °C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in Table 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997). The alignment was manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). Maximum parsimony analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences.

Table 1. Sources of sequences for molecular analysis

Species	Voucher specimen	Geographical origin	ITS
Terfezia boudieri Chatin	mot08	Algeria	AF276673
	DDtb1	Tunisia	GU474809
Tuber aestivum Vittad.	E1	Italy	AF516788
	E17	Italy	AY226042
T. borchii Vittad.	GB62	Italy	HM485342
	GB45	Italy	HM485344
T. excavatum Vittad.	Texc-eu02	France	DQ329362
	BM100	Spain	FJ748899
?T. excavatum	HKAS 52006	China	GQ217540
T. fulgens Quél.	M2435	Italy	HM485358
	SFI: TUBFUL/041008B	Slovenia	FN433150
T. magnatum Picco	Tm4	Italy	AJ586308
	—	Italy	EU807975
T. melanosporum Vittad.	A59	France	AF106878
	Tm13	France	AF132501
T. mesentericum	TmsW095-W029 (UPS)	Sweden	AJ888047
	TmsW088-W047 (UPS)	Sweden	AJ888044
T. neoexcavatum	**BJTC FAN184 (Holotype)**	**China**	**JX458715**
T. pseudoexcavatum	Tpse-yn05	China	DQ329374
T. pseudohimalayense	BJTC FAN120	China	JX458716
T. rufum Picco	Morphotype 5	Italy	AY940646
	1506	Italy	AY112894
T. sinoexcavatum	BJTC FAN130 (Holotype)	China	JX458717
	BJTC FAN166	Chinan	JX458718

TBR branch swapping was employed. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist & Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. Two sequences derived from *Terfezia boudieri* were used as outgroup.

Results

Molecular phylogenetics

480 of 688 characters were found to be parsimony-informative. The maximum parsimony analysis of sequences resulted in one most parsimonious tree (Fig. 1) with a length (TL) = 1422 steps, consistency index (CI) = 0.6582, retention index (RI) = 0.8002, homoplasy index (HI) = 0.3418, and rescaled consistency index (RC) = 0.5267.

The phylogenetic analysis (Fig. 1) reveals that the *Tuber* species with excavated ascomata are scattered in different clades with strong support. The new species *T. neoexcavatum* clusters in a clade with other three other

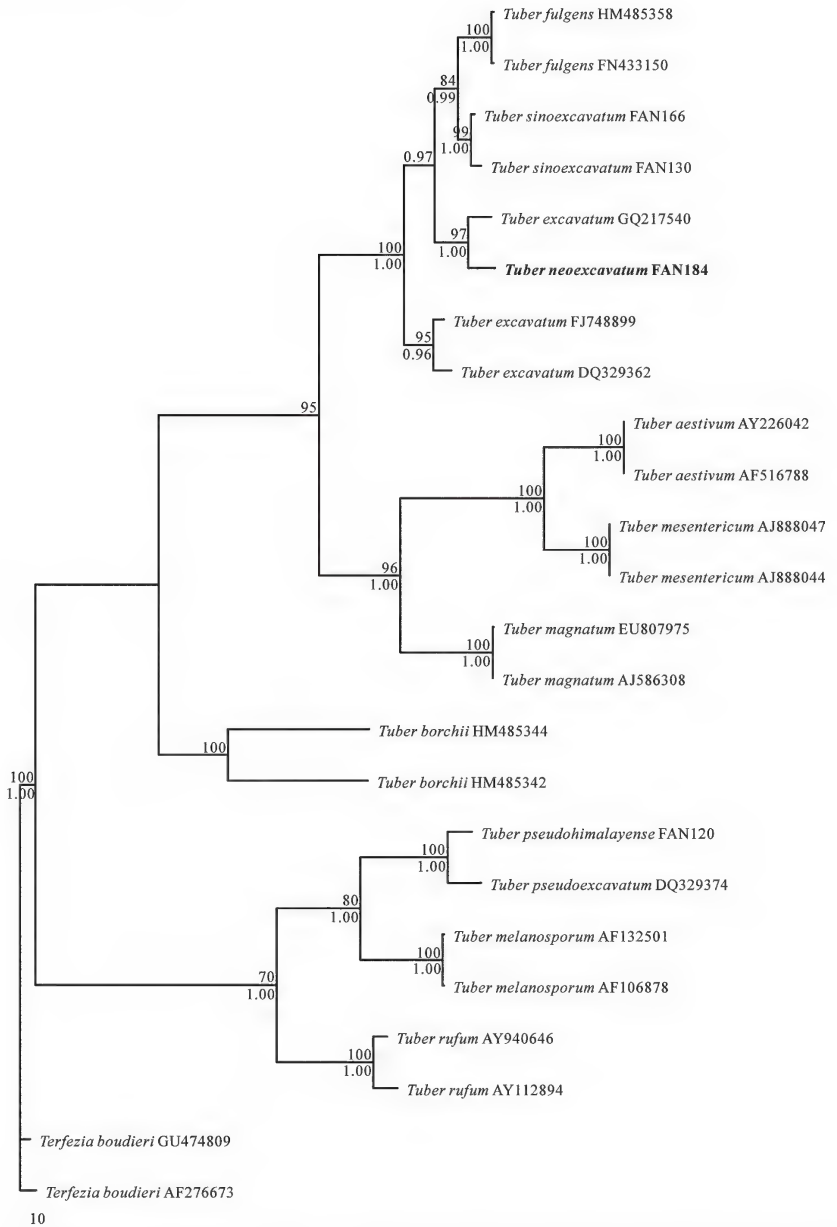

FIG. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences of some *Tuber* species with real excavated ascomata, using *Terfezia boudieri* as outgroup. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches. Bayesian posterior probabilities are estimated and clades with PP>0.95 (95%) are marked under the branches.

excavated species: *T. excavatum*, *T. fulgens*, and *T. sinoexcavatum*. Furthermore, four strongly supported subclades (each represented by two sequences) correlate exactly with individual taxa as defined using morphological methods, indicating that the four excavated species are closely related but distinct. The other two excavated species are in two separate clades: *T. pseudohimalayense* grouping with *T. melanosporum* and *T. mesentericum* with *T. aestivum*.

Taxonomy

Tuber neoexcavatum L. Fan &Yu Li, **sp. nov.** FIG. 2

MYCOBANK MB 801128

Differs from other *Tuber* species by its brown ascoma with a distinctly verrucose surface and prosenchymatous peridium.

TYPE: China. Yunnan Province, Baoshan City, in soil under forest of *P. yunnanensis* Franch., 28 Dec. 2011, Jin-Zhong Cao 511 (**Holotype**, BJTC FAN184).

ETYMOLOGY: *neoexcavatum* (Lat.), referring to the similarity to *Tuber excavatum*.

ASCOMATA hypogenous, subglobose, 3–4 cm in diam., with typical basal cavities (excavated ascomata), covered with distinctly verrucose warts on the surface, yellow brown to brown with shadow of olive green; interior cavity surface covered with many fine, irregular scales and large warts, pale white to yellow-white when fresh. Odor and flavour not recorded. PERIDIUM 250–350 μm thick including the 100–150 μm high verrucose warts, prosenchymatous, composed of interwoven hyphae, light brown and slightly thick-walled towards the outside, 3–6 μm in diam., hyaline and thin-walled towards the inside, 2.5–5 μm in diam. GLEBA yellow brown to brown, with the shadow of olive green at maturity, veins white to cream white, more or less radiate from the cavity. ASCI subglobose or long oval, hyaline, thin walled, 75–100 × 62.5–80 μm, with a short stalk, 2–4-spored. ASCOSPORES elliptic or long elliptic, occasionally broadly elliptic, hyaline at first, light brown or brown at maturity, reticulate ornamentation on the surface, 30–47.5 × 25–30 μm excluding the ornamentation, meshes 5–6 μm deep, 2–4 across the spore width.

ADDITIONAL SPECIMEN EXAMINED — **CHINA. SICHUAN PROVINCE**, HUIDONG COUNTY, in soil under forest of *P. armandii* and *P. yunnanensis*, 3 Nov. 2006, Juan Chen 411 (HKAS 52006, as *T. excavatum*)

COMMENTS — Ascomata of *Tuber neoexcavatum* resemble those of *T. pseudohimalayense* (= *T. pseudoexcavatum*), including the color and small distinct surface warts, but *T. neoexcavatum* is easily distinguished by its spino-reticulate ornamented ascospores. *Tuber excavatum, T. fulgens,* and *T. sinoexcavatum* also produce similar ascomata, but *T. excavatum* is distinguished by its pale and smooth or finely papillar ascomata and *T. fulgens* and *T. sinoexcavatum* produce globose or subglobose ascospores. The European species, *T. mesentericum,* is also excavated but differs from *T. neoexcavatum* in its blackish ascomata that

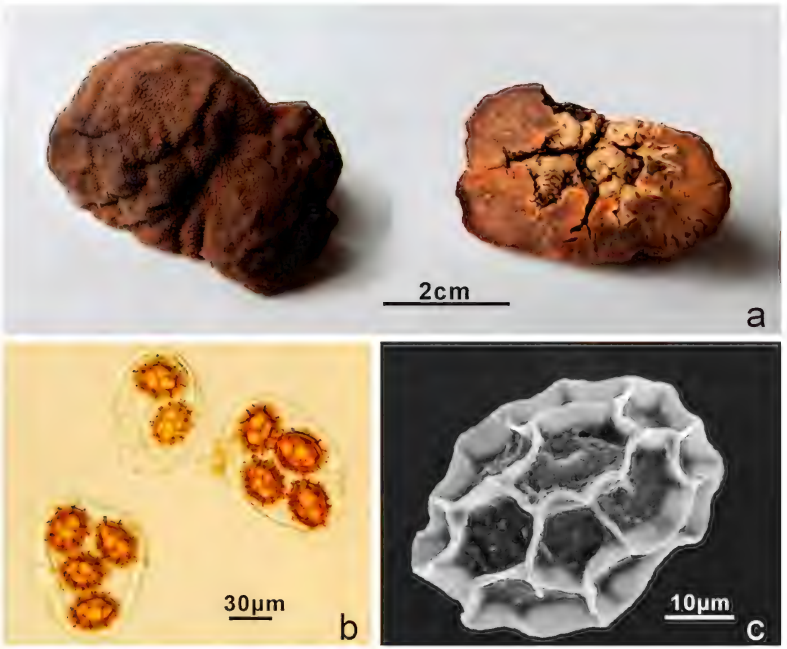

Fig. 2. *Tuber neoexcavatum* (BJTC FAN184, holotype). a Ascomata; b Asci and ascospores observed under light microscope; c Ascospore observed under SEM.

are completely covered with large pyramid warts. In addition, the peridium structure in *T. neoexcavatum* is typically prosenchymatous, which is completely (including the verrucose warts) composed of interwoven hyphae. The ITS rDNA phylogeny (Fig. 1) places *T. neoexcavatum* sequences within an evidently independent clade (BP = 97, PP = 1.00) and clearly supports the new species as independent.

Chen et al. (2008) reported *T. excavatum* in China based on specimen HKAS 52006 collected in 2006 from Sichuan Province (also see Cao 2010). However, our ITS rDNA sequence analysis of HKAS 52006 and other excavated *Tuber* species (Fig. 1) places sequence GQ217540 (from HKAS 52006) in a clade with our *T. neoexcavatum* but not with the *T. excavatum* sequences from France and Spain. In examining HKAS 52006, we could find no ascomal surface and peridial differences between the specimen of Chen et al. (2008) and the type specimen of *T. neoexcavatum*. We therefore redetermine HKAS 52006 as *T. neoexcavatum*.

As all other reports of *T. excavatum* in China are not linked to specimens, there is no firm evidence for *T. excavatum* being present in China. Whether *T. excavatum* occurs in China or not needs further investigation.

Tuber sinoexcavatum L. Fan & Yu Li, Mycotaxon 116: 352 (2011)

Specimens examined — **China. Sichuan Province**, Panzhihua City, under the soil of *Pinus* sp., 20 Dec. 2007, De-fu Liu (Holotype, BJTC Fan130); **Yunnan Province**, Huizhe County, in soil under forest, 12 Nov. 2011, Shao-ping Li 005 (BJTC Fan166).

Distribution: China: Sichuan Province; Yunnan Province.

Comments—*Tuber sinoexcavatum* was originally described from one collection (BJTC Fan130), but in 2011 an additional specimen (BJTC Fan166) was found in Yunnan Province, China. The sequence analyses (Fig. 1) support the two specimens as conspecific. It should be pointed out that the ascospores are near regularly globose in the type specimen, but in the new specimen about half the ascospores are subglobose or broadly elliptic. This phenomenon also occurs in *T. latisporum* Juan Chen & P.G. Liu and *T. subglobosum* L. Fan & C.L. Hou (Fan et al. 2013). The *T. latisporum* ascospores are broadly elliptic to subglobose, but also sometimes globose in some ascomata in the same collection, while in some ascomata *T. subglobosum* may occasionally produce a large proportion of broadly elliptic ascospores rather than typical subglobose ones.

Tuber sinoexcavatum is morphologically similar to the European species *T. excavatum*, which, however, produces elliptic ascospores. Furthermore, the peridium in *T. sinoexcavatum* is normally covered with fine papillary warts while *T. excavatum* produces smooth or only finely scurfy warts (Pegler 1993, Riousset et al. 2001). Another European species, *T. fulgens,* also produces globose ascospores, but its ascoma is brightly colored and a small hole opens to the outside (Lange 1956, Riousset et al. 2001), unlike the large opening in *T. sinoexcavatum*. Here also the ITS rDNA sequence analyses strongly support three separate species (Fig. 1).

Tuber pseudohimalayense G. Moreno, Manjón, J. Díez & García-Mont., Mycotaxon 63: 218 (1997)

= *T. pseudoexcavatum* Y. Wang, G. Moreno, Riousset, Manjón & G. Riousset, Cryptog. Mycol. 19(1–2): 115 (1998)

Specimens examined — **China. Sichuan Province**, Huizhe County, in soil under trees, 19 Oct. 2003, Juan Chen 127 (HKAS 44346); 3 Nov. 2006, Juan Chen 412 (HKAS 52010); 5 Nov. 2006, Juan Chen 416 (HKAS 52017); 20 Oct. 2003, Juan Chen 134 (HKAS 44325); 19 Oct. 2003, Juan Chen (HKAS 44346); in soil under *P. armandii*, 15 Dec. 2008, Li Fan 020 (BJTC FAN120); **Yunnan Province**, Kunming City, in soil under forest, 25 Nov. 2002, Zhu-liang Yang 3603 (HKAS 41313); 8 Nov. 2004, Juan Chen 234 (HKAS 47619); 8 Nov. 2002, Juan Chen 287 (HKAS 49744); 9 Mar. 2003, Xing-hua Wang 1477 (HKAS 42399); Jinning County, in soil under trees, 20 Sep. 2006, Juan Chen 427 (HKAS 52032); Yongsheng County, in soil under trees, 29 Oct. 2006, Juan Chen 407 (HKAS 52027); Juan Chen 408 (HKAS 52036).

Distribution: China: Sichuan Province; Yunnan Province.

Comments—Moreno et al. (1997) described *T. pseudohimalayense* for a black truffle with spino-reticulate ornamented ascospores. Although the type

specimen was found in a Spanish truffle market, the authors suspected it was probably imported from China (Moreno et al. 1997). The next year, Wang et al. (1998) published *T. pseudoexcavatum* to accommodate a very common black truffle species in China. It and *T. pseudohimalayense* produce identical ascospores, but *T. pseudohimalayense* is characterized by a distinct cavity at the ascomal base. Manjón et al. (2009) and Chen & Liu (2011) concluded that the two 'species' were definitely conspecific based on their analysis of molecular data. As the earlier published name, *T. pseudohimalayense* has priority over *T. pseudoexcavatum*. We have found it is widely distributed in Yunnan and Sichuan provinces of southwest China during autumn and early winter, and Song (2005) has cited it from Hunan Province. Its spino–reticulate ascospores and 1–8-spored asci separate *P. pseudohimalayense* from all other excavated *Tuber* species.

Discussion

Six total species of *Tuber* with excavated ascomata have been described thus far, including three species from China. As noted above, the European species *T. excavatum* has not yet been confirmed in China.

The ITS rDNA–based phylogeny supports four closely related species — *T. excavatum*, *T. fulgens*, *T. sinoexcavatum, T. neoexcavatum* — within a well-supported clade and shows that *T. pseudohimalayense* is close to *T. melanosporum* and *T. mesentericum* is close to *T. aestivum*. Although *Tuber* species with excavated ascomata appear morphologically similar, however, their rDNA sequence analyses imply that they are definitely polyphyletic.

Key to *Tuber* species with excavated ascomata

1a. Ascomata blackish; surface with pyramidal warts ..... *T. mesentericum*
1b. Ascomata not blackish; surface smooth, verrucose, or with small warts 2
2a. Ascomata distinctly verrucose or with small warts 3
2b. Ascomata smooth or minutely verrucose 4
3a. Ascospores with spino–reticulate ornamentation ..... *T. pseudohimalayense*
3b. Ascospores with reticulate ornamentation ..... *T. neoexcavatum*
4a. Ascospores mostly elliptic ..... *T. excavatum*
4b. Ascospores mostly globose or subglobose 5
5a. Ascomata golden brown, opening to outside by a small hole ..... *T. fulgens*
5b. Ascomata yellow brown to light brown, typically excavated ..... *T. sinoexcavatum*

Acknowledgments

We are grateful to Prof. Guozhong Lü and Dr. Ian Hall for reviewing the manuscript. The study was supported by the National Natural Science Foundation of China (No. 31270058, 30770005), the Beijing Natural Science Foundation (No. 5072006). We wish

to extend a special thanks to the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences, for providing specimens.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.165

Volume 124, pp. 165–171 *April–June 2013*

A new species and a new record of the genus *Entoloma* from China

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Abstract —Two omphalinoid *Entoloma* species were collected from Shandong Province in China. *Entoloma shandongense* is described as new to science, and *E. rusticoides* is recorded for the first time in China. Detailed descriptions and illustrations of the two species are provided. In addition, molecular phylogenetic analysis based on ITS sequences is also presented.

Key words —*Entolomataceae*, new taxon, morphology, taxonomy

Introduction

More than one thousand species of *Entoloma* have been described worldwide (Kirk et al. 2008). Major works on this genus have been published in the past twenty years (Largent 1994, Noordeloos 2004, Noordeloos & Hausknecht 2007, Gates & Noordeloos 2007, Horak 2008, Noordeloos & Gates 2009, 2012). In China, 137 species of *Entoloma* have been recorded, including several new taxa and new records in recent systematic studies (Li et al. 2008, 2009; He et al. 2010, 2011, 2012, 2013).

Recently, two omphalinoid *Entoloma* species were collected in Shandong Province. One with a blue pileus is described as new to science, and the other represents a first record for China. Morphological characters and the results of ITS sequence analyses are provided for both species.

Materials & methods

Materials were collected from Shandong province, north China and were deposited in the Herbarium of Mycology of Ludong University (HMLD). Morphological characters of the two species were described and illustrated using traditional taxonomic methods.

For morphological studies, color notations follow Kornerup & Wanscher (1978). Microscopic characters were examined using water to observe the color of the tissues,

5% KOH solution as a mounting solvent, 1% Congo Red solution as a staining agent, and Melzer's reagent to detect amyloidy. At least 20 basidiospores, basidia, and cystidia per specimen were measured.

The molecular confirmation was derived from ITS sequences. DNA was extracted according to Sun et al. (2009). ITS rDNA was amplified using primers ITS1 and ITS4. Amplification was performed in 25 μL volumes containing 0.5 μL template DNA, 2.5 μL PCR-buffer (10×), 2 μL Mg2+ (25 mmol/L), 0.2 μL Taq enzyme (5 U/μL), 0.5 μL dNTP mix (10 mmol/L), 0.5 μL of each primer and 17.8 μL ddH_2O. The reaction was carried out with initial denaturation (94°C, 3 min), 35 cycles by the following conditions: denaturation (94°C, 50s), annealing (50°C, 30s), extension (72°C, 50s), final extension (72°C, 10min). The PCR amplification products were sequenced by Sangon Co., Shanghai, China. The target gene sequences were checked using Bioedit (Version 7.0.9; Hall 1999). The final BLASTN was done in Genbank.

Entoloma shandongense T. Bau & J.R. Wang, **sp. nov.** FIG. 1

MycoBank MB 802463

Differs from *Entoloma ater* by its blue pileus and its smaller basidiospores.

TYPE: China, Shandong Province, Dezhou City, Botanical Garden of Dezhou, 2 September 2011, Jian-Rui Wang (**Holotype**, HMLD 1796; GenBank, KC257440).

ETYMOLOGY: *shandongense* refers to the type locality, Shandong Province.

BASIDIOMATA small, omphalinoid. PILEUS 10–18 mm broad, hemispherical, depressed in the center to infundibuliform, not hygrophanous, slightly translucent-striate almost to center, smooth, mostly blue with somewhat purple tinge (22B5–22B4) over the whole surface, slightly deeper (21C5–21C6) or deep dull blue-violet (20C5–20C6) in the center, tomentose. LAMELLAE decurrent or adnate with short decurrent tooth, 1.5–2 mm broad, distant, pale pinkish beige (7A1–7A2) to pale pinkish red (11A1–11A2); margin entire. LAMELLULAE present, 2-3 tiers. CONTEXT thin, whitish to pale brown. STIPE central to somewhat eccentric, 10–15 mm in length, 1–2 mm in width, cylindrical to subclavate, gradually narrowing towards base, concolorous with lamellae, pale beige pink, light beige to white at base. ODOR AND TASTE not distinctive.

BASIDIOSPORES 5.6–7.2 × (7.5–)8.3–10(–10.5) μm, Q = 1.3–1.5, with obvious apiculus, 5–8-angled in side view, thick-walled. BASIDIA 29–36 × 8–10 μm, clavate, 4-spored, hyaline, thick-walled when mature, homogeneous or with granular contents. PLEUROCYSTIDIA & CHEILOCYSTIDIA absent. LAMELLAR TRAMA regular, made up of cylindrical, narrow elements 30–70 × 8–12 μm. PILEIPELLIS a trichoderm of cylindrical hyphae, often narrower than lamellar hyphae, with terminal elements 30–70 × 5–8 μm, pigment intracellular, plasmatic, dark blue with somewhat purple tinge, sometimes forming clots, which dissolve in 5% KOH. SUBPELLIS composed of subhyaline or very pale blue plasmatic pigment. PILEITRAMA made up of cylindrical elements, hyaline, thin-walled. STIPITIPELLIS composed of hyphae 5–8 μm in diameter, hyaline,

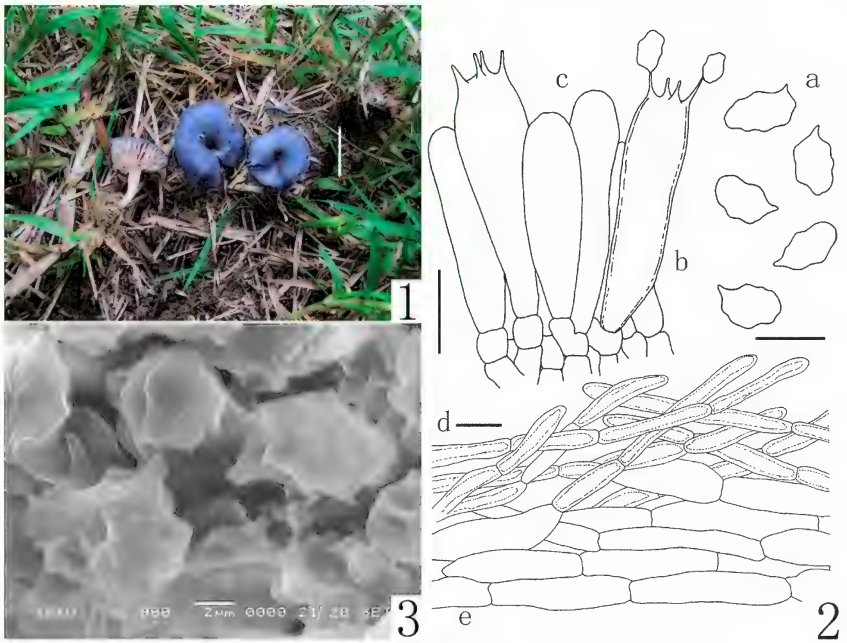

Fig. 1. *Entoloma shandongense* (holotype): 1. Basidiomata (in situ).
2. Line drawings: a. Basidiospores; b. Basidia; c. Young basidia; d. Pileipellis; e. Pileitrama.
3. SEM: Basidiospores. Scale bars: 1 = 10 mm; 2 = 10 μm.

thin-walled, without intracellular pigment. Stipe trama regular, made up of longitudinally cylindrical hyphae up to 5–10 μm in diameter. Caulocystidia absent. Oleiferous hyphae scattered in lamellar trama and pileal trama. Brilliant granules abundant in all tissues. Clamp connections absent.

Ecology — on soil in *Poa pratensis* grassland.

Commentary — The main characteristics of *Entoloma shandongense* are the omphalinoid blue pileus, the many-angled to nodulose spores, and the absence of cystidia and clamp connections.

There are many species with a blue pileus in *Entoloma*, but they are rarely omphalinoid. Only *E. ater* (Hongo) Hongo & Izawa (Hongo 1958) resembles *E. shandongense* in its small omphalinoid basidiomata and grassland association, but the pileus of *E. ater* is often purplish and its basidiospores are larger. Some *Entoloma* species with blue caps lacking the violet hues have recently been described from China (Li et al. 2009, He et al. 2012), e.g., *E. azureosquamulosum* T.H. Li & Xiao Lan He, *E. subaltissimum* T.H. Li & Chuan H. Li, and *E. dinghuense* T.H. Li & Chuan H. Li. *Entoloma azureosquamulosum*

differs from *E. shandongense* in its convex or conical cap and possession of hymenial cheilocystidia and caulocystidia on the stipe. The spores of *Entoloma subaltissimum* are isodiametric and those of *E. dinghuense* are quadrate, neither heterodiametric as in *E. shandongense*.

Other eastern Asian species with blue-violet pilei (e.g., *E. cyanonigrum* (Hongo) Hongo, *E. violaceum* Murrill, *E. glutiniceps* (Hongo) Noordel. & Co-David, *E. kujuense* (Hongo) Hongo) all differ in larger size (pilei often >2 cm broad) and a dark purplish (not blue) coloration. In addition, *E. cyanonigrum* spores are larger (10.4–11.7 × 6.5–9.1um; Katumoto 2010) and *E. glutiniceps* spores are isodiametric (7–8 µm diam.; Co-David 2009), distinguished from the smaller ellipsoid spores of *E. shandongense*. Finally, pleurocystidia and cheilocystidia, absent in *E. shandongense,* are present in both *E. kujuense* (Hongo 1985) and *E. violaceum* (Murrill 1917).

Entoloma rusticoides (Gillet) Noordel., Persoonia 11(2): 150, 1981. FIG. 2

BASIDIOMATA small, mycenoid or omphalinoid. PILEUS 8–14 mm broad, convex to hemispherical, sometimes with a small papilla, becoming depressed to umbilicate in the center, hygrophanous, translucent-striate almost to center, smooth, dark gray to umber brown, light beige brown when dry, finely tomentose, center always whitish fibrillose-scaly, margin incurved and undulating. LAMELLAE pale beige to pale brown-pink, decurrent; margin entire. CONTEXT thin, cream-colored to whitish. STIPE central, 10–15 mm in length, 1–1.5 mm in width, cylindrical to subclavate, surface smooth, gray to brown, whitely tomentose at base. ODOR faintly herbaceous, TASTE mild.

BASIDIOSPORES (8.2–)8.5–10.5(–11.0) × (7.6–)8.0–10.3(–11.7) µm, ellipsoid to sub-isodiametric, 5–6-angled in side view, thick-walled, straw coloured. BASIDIA 28–41 × 9.5–10.5(–12) µm, cylindrical to clavate, 2 or 4-spored, hyaline, thick-walled when mature, clampless. PLEUROCYSTIDIA & CHEILOCYSTIDIA absent. LAMELLAR TRAMA regular, made up of cylindrical elements, 50–100 × 5–15 µm. PILEIPELLIS made up of cylindrical elements 8–20 µm in diameter, septa without clamps, occasional hyphal ends slightly inflated, up to 30 µm in diameter, thin-walled, without clamp connections, externally encrusting pigments brown. STIPITIPELLIS composed of clampless hyphae 8–15 µm, thin-walled, encrusting pigments brown. STIPE TRAMA regular, made up of longitudinal cylindrical hyphae, up to 5–15 µm in diameter, without clamp connections. CAULOCYSTIDIA absent.

ECOLOGY — in soil alongside paths or associated with *Poa pratensis* or *Cynodon dactylon* grasslands.

SPECIMENS EXAMINED — **CHINA, SHANDONG PROVINCE**, HEZE: Zhaowanghe Garden, 7 August 2011, Jian-rui Wang (HMLD 1442; GenBank, KC257438); LIAOCHENG: Dongchanghe, 1 September 2011, Jian-rui Wang (HMLD 1490; GenBank, KC257441).

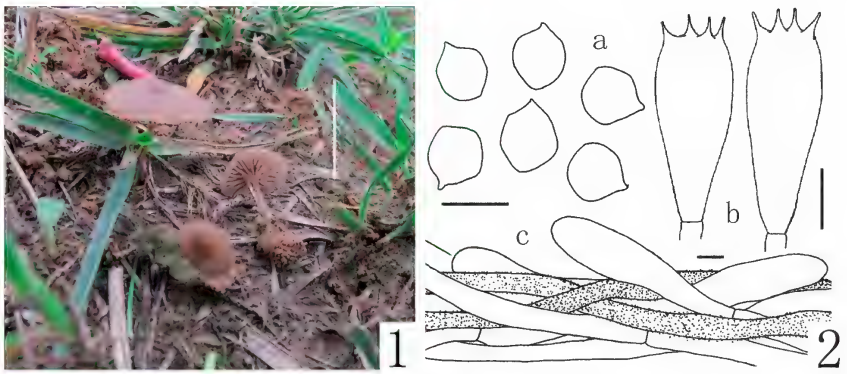

Fig. 2. *Entoloma rusticoides* (HMLD1442): 1. Basidiomata (in situ).
2. Line drawings: a. Basidiospores; b. Basidia; c. Pileipellis.
Scale bars: 1 = 10 mm; 2 = 10 μm.

Commentary — *Entoloma rusticoides* is similar to *E. rhodocylix* (Lasch) M.M. Moser, *E. phaeocyathus* Noordel., and *E. flocculosum* (Bres.) Pacioni in its omphalinoid shape and sub-isodiametric spores. However, *E. rhodocylix* (Moser 1978) and *E. phaeocyathus* (Noordeloos 1985) both have cheilocystidia in the lamellar edge, and *E. flocculosum* (Pacioni 1987) differs in its non-striate, densely floccose-scaly pileus.

Discussion

The morphology-based taxonomic differences of *Entoloma shandongense, E. rusticoides,* and related species have been confirmed by ITS sequence analyses.

The ITS sequence of the *E. shandongense* holotype is similar to those of *E. platyphylloides* (Romagn.) Largent (GenBank JF908003) and *E. undatum* (Gillet) M.M. Moser (GenBank JF908007), with higher query coverage values (≤99%) and max identity (>90%) than other GenBank sequences. However, the three species obviously differ in fruitbody and spore color, shape, and size. *Entoloma platyphylloides,* which occurs in Europe, has a brownish pileus >2 cm in diameter (Kühner & Romagnesi 1954), while *E. undatum* has a gray-white pileus and sometimes possesses cheilocystidia (Moser 1978).

Two ITS sequences from the two *E. rusticoides* specimens are close to those of *E. infundibuliforme* Hesler (GenBank HQ179671), *E. sericeoides* (J.E. Lange) Noordel. (GenBank JF908006), and *E. defibulatum* Arnolds & Noordel. (GenBank JX454928), with higher query coverage values (≤98%) and max identity (≤93%) than other GenBank sequences. However, the spores of *E. defibulatum* (rounded-angular; Arnolds & Noordeloos 1979) and

E. sericeoides (Noordeloos 1980) are smaller than in *E. rusticoides*. *Entoloma infundibuliforme* has a squamulose pileus and brown cheilocystidia (Hesler 1967) not found in *E. rusticoides*.

Acknowledgments

We thank Dr. T.H. Li (Guangdong Institute of Microbiology, China) and Dr. Genevieve Gates (School of Agricultural Science, University of Tasmania, Australia) for their reviews of the manuscript. We also thank Dr. X.L. He (Sichuan Academy of Agricultural Sciences, China) for providing valuable suggestions and references in the study. Financial support for this work was provided by Changjiang Scholars and Innovative Research Team in University (No. IRT1134).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.173

Volume 124, pp. 173–188 *April–June 2013*

Russula atroaeruginea and *R. sichuanensis* spp. nov. from southwest China

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Abstract — Two new species of *Russula* are described from southwestern China based on morphology and ITS1-5.8S-ITS2 rDNA sequence analysis. *Russula atroaeruginea* (sect. *Griseinae*) is characterized by a glabrous dark-green and radially yellowish tinged pileus, slightly yellowish context, spores ornamented by low warts linked by fine lines, and numerous pileocystidia with crystalline contents blackening in sulfovanillin. *Russula sichuanensis*, a semi-sequestrate taxon closely related to sect. *Laricinae*, forms russuloid to secotioid basidiocarps with yellowish to orange sublamellate gleba and large basidiospores with warts linked as ridges. The rDNA ITS-based phylogenetic trees fully support these new species.

Key words — taxonomy, *Macowanites*, *Russulales*, *Russulaceae*, *Basidiomycota*

Introduction

Russula Pers. is a globally distributed genus of macrofungi with colorful fruit bodies (Bills et al. 1986, Singer 1986, Miller & Buyck 2002, Kirk et al. 2008). As ectomycorrhizal symbionts, they play an important beneficial role in forest ecosystems (Richardson 1970, Bills et al. 1986, Villeneuve et al. 1991, Claridge & May 1994, Buyck et al. 1996, Gardes & Bruns 1996). China has a long history of using *Russula* mushrooms as traditional food and medicine. Twenty-two medicinal species and 82 edible species have been reported in the country (Ying et al. 1982, 1987; Wang & Liu 2002; Yang 2002; Wang et al. 2004; Dai et al. 2009; Li et al. 2010a), and their ectomycorrhizal capacity, population genetics, and mycochemistry have been studied in recent years (Gao et al. 2001;

Tan et al. 2001, 2004; Liang et al. 2004; Liu 2004, 2005; Li et al. 2010b; Zhao et al. 2010). So far, only 14 new species and three new varieties have been reported from China (Singer 1935; Chiu 1945; Ying 1983, 1989; Bi & Li 1986; Zang & Yuan 1999; Wen & Ying 2001; Wang et al. 2009; Li et al. 2011, 2012). Although southwestern China represents one of the world's biodiversity "hotspots" and has a high diversity of macrofungi (Yang & Zang 2003; Dai et al. 2004, 2007; Dai & Yuan 2008), only a few russulacean species have been reported in this region and the adjacent Himalayas (Wang & Liu 2009, 2010; Das et al. 2010; Buyck & Atri 2011; Das & Verbeken 2011; Wang et al. 2012). During a systematic survey of *Russula* species in southwestern China from 2004 to 2009, two interesting species were found, which we propose here as new species.

Materials & methods

Specimens were photographed, and the macroscopic characteristics of fresh mature fruit bodies were recorded under daylight in the field. Specimens were tested chemically in sulfovanillin (SV) solution. They were then kept 50–60 °C until completely dried and were subsequently deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Color names and codes were assigned based on Ridgway (1912). Tissues of dried specimens were immersed in 5% KOH for 10–20 seconds to prepare for microscopic observation in Congo Red using a Nikon E80i microscope with a 100× oil immersion objective lens. Basidiospores were observed and measured in Melzer's reagent. Spore measurements do not include apiculus and ornamentation, and sterigma lengths were excluded from basidium measurements. Sections from the pileipellis were anticlinally cut through the pileus centre to the margin. Cystidia with incrustations were observed in distilled water. Scanning electron microscopic images were captured with an FEI Quanta 200 microscope. Microscopic observation and statistical citation of measure technicalities follow Yang (2000) and Wang et al. (2009).

Protocols for DNA extraction, PCR, and sequencing followed those in Li et al. (2012). The internal transcribed spacer (ITS) regions were amplified with the primer pairs ITS1/ITS4 (Gardes & Bruns 1993). PCR products were purified with the Bioteke DNA Purification Kit (Bioteke, Beijing, China). The ITS regions were sequenced with the ABI 3730 DNA analyzer and ABI Bigdye 3.1 terminator cycle sequencing Kit (BGI, Beijing, China), and new sequences were deposited in GenBank (see Fig. 5 for accession numbers). Sequences of representative and closely related *Russula* taxa, with an emphasis on samples of the russuloid to secotioid forms (Eberhardt 2002, Miller & Buyck 2002, Lebel & Tonkin 2007), were selected from GenBank and included in this analysis. Assembly and editing of sequences of each region were performed with Clustal X and BioEdit (Thompson et al. 1997, Hall 1999) and were manually adjusted when necessary. Some poorly aligned terminal sites were excluded from further analysis.

Based on previous results (Lebel & Tonkin 2007), *Albatrellus ovinus* (Schaeff.) Kotl. & Pouzar was chosen as outgroup (Ryman et al. 2003). Sequences of datasets including the ITS1-5.8S-ITS2 region were analyzed with maximum parsimony (MP) and Bayesian analysis (BA) methods.

MP analysis of the phylogenetic relationships were performed in PAUP* v.4.01 (Swofford 2004). Gaps in alignment were treated as missing data, and all sites were treated as unordered and unweighted. The tree bisection-reconstruction (TBR) algorithm was performed using with the heuristic search option, and bootstrap analysis was conducted with 1000 replicates (Felsenstein 1985). Consistency index (CI), retention index (RI), and tree length (TL) were also calculated. Trees were displayed with Treeview 1.6.6 (Page 1996).

Bayesian analysis was carried out in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The best-fit model of Bayesian posterior probabilities (PP) were calculated with the Markov Chain Monte Carlo (MCMC) algorithm (Larget & Simon 1999). One cold and three heated Markov chains were run for 2,000,000 generations, the trees were sampled every 100th generation, and the run was terminated once the average standard deviation of split frequencies fell below 0.01. A discard of 25% trees was set in the Burn-in option (Hall 2004).

Taxonomy

Russula atroaeruginea G.J. Li, Q. Zhao & H.A. Wen, **sp. nov.** FIGS 1–2

MYCOBANK MB804644

Differs from *Russula aeruginea* by its non-fading green pileus lacking scattered rusty spots and whitish to pale cream spore print.

TYPE: China. Sichuan Province, Daofu County, 25.VII.2007, Z.W. Ge 1540 (**Holotype**, HKAS53626, GenBank JX391970)

ETYMOLOGY: *atroaeruginea* (Latin) = dark green. The epithet refers to the dark-green basidioma pileus.

BASIDIOMATA small to medium sized. PILEUS 3–7 cm in diam., first hemispherical, then convex to applanate, sometimes slightly depressed above the stipe, blackish-green tinged, intermixed with radial greenish yellow fringe, Dull Blackish Green (XLI29′′′m) at centre, Sepia (XXIX17′′m) when dry, never viscous; margin incurved first, straight when mature, sometimes undulate, not striate, Meadow Green (VI35k) to Antique Green (VI33m) with yellowish tinge of Oil Yellow (V25i) towards the margin, Verona Brown (XXIX13′′k) when dry. LAMELLAE 10–15/cm at the edge, ≤7 mm wide, adnate to almost free, forked around the stipe, occasionally near the pileus margin, not interveined, cream to yellowish, Cream Color (XVI19′f), brittle; edge even, slightly narrowing towards the pileus margin; lamellulae occasional. STIPE 4–6 × 1–2 cm, central to slightly eccentric, smooth, not pruinose, cylindrical, ventricose toward the base, without annulus, whitish, often with pale-greenish tinge, part turning pale yellow when aged, sometimes with rusty spots, first unchanging, stuffed, hollow when mature. CONTEXT 3–5 mm thick from the lamellae attachment to the stipe, compact under pileus, white to whitish, no color change when bruised, turning slightly pale yellowish in age; taste mild; no distinct odor. SPORE PRINT whitish to pale cream (Romagnesi Ib–IIa).

edge sterile. Subhymenium 15–20 µm thick, composed of slender cylindrical cells 4–7 µm wide under basidia and inflated cells (15–)20–35(–45) µm in diam. next to trama. Pileipellis a trichoderm 200–300 µm thick, composed of 4–6 µm thick, slender hyaline hyphae without intracellular pigmentation; pileocystidia numerous, 40–70 µm long, sometimes inflating up to 7–10 µm wide, fusiform, with crystal contents but no septa, sharply blackened in SV. Stipitipellis not well developed, a cutis composed of thin-walled, septate, cylindrical hyphae 3–6 µm wide; caulocystidia absent. Clamp connections absent in all tissues.

Ecology & distribution: Single or gregarious in conifer forest (*Picea* spp.) in July and August in China (Sichuan, Xizang, and Yunnan).

Additional specimens examined: **China, Sichuan Province**, Daofu County, 25.VII.2007, Z.W. Ge 1532 (HKAS53618); **Yunnan Province**, Yulong, Laojunshan, 9.VIII.2008, Q. Zhao 8238 (HKAS55220); **Xizang Autonomous Region**, Changdu County, Zhuge Village, 31°04′N, 96°58′E, alt. 4200 m, 7.VIII.2003, Z.L. Yang 4305 (HKAS45684).

Comments: *Russula atroaeruginea* is characterized by its dark-green intermixed with radially yellowish-tinged (striate) pileus, whitish to pale-cream spore print, and basidiospores with thin low fine ornamentations (<1.0 µm tall). The dark-green pileus contrasting with the whitish (often green-tinged) stipe is a good field character, although it is sometimes difficult to distinguish this species against from the green forest surroundings. This species should be placed in the *Russula* sect. *Griseinae* (Jul. Schäff.) Romagn. based on its mild-flavored context, pileipellis lacking primordial hyphae and red pigments but with typical multiseptate (often subulate) hyphal extremities, and unicellular pileocystidia that blacken in SV.

Romagnesi (1967) recognized 5 greenish tinged species in sect. *Griseinae*: *R. pseudoaeruginea* (Romagn.) Kuyper & Vuure, *R. aeruginea* Fr., *R. medullata* Romagn., *R. stenotricha* Romagn., and *R. anatina* Romagn. The morphologically similar *R. aeruginea*, *R. anatina*, *R. cyanoxantha* f. *peltereaui* Singer, *R. medullata*, and *R. pseudoaeruginea* have been reported in eastern Asia (Tai 1979, Ying et al. 1982, Imazeki & Hongo 1989, Ying 1989, Shimono et al. 2004). *Russula pseudoaeruginea*, however, has a gray-tinged green olive or somewhat cream and pale-brown intermixed pileus and an ochreous-cream spore print (Romagnesi 1967, Sarnari 1998); it often grows in temperate to boreal deciduous forests dominated by *Tilia* and *Quercus* spp. (Romagnesi 1967, Hansen & Knudsen 1992), while *R. atroaeruginea* grows in conifer forests dominated by *Picea* spp. in alpine subtropical regions.

Another taxon superficially resembling *R. atroaeruginea* is *R. aeruginea*, a very common species originally described from Europe which grows in subalpine coniferous to mixed forests containing *Betula*, *Pinus*, and *Picea*. However, *R. aeruginea* has a green-tinged pileus with a darker pileus centre, whitish

Fig. 1: *Russula atroaeruginea*: a. HKAS 45684; b. HKAS 53626 (Holotype)

stipe with rusty spots, a slightly acrid taste, cream-colored spore deposits, and spores with many fine lines formed by connected low warts (Schaeffer 1952, Romagnesi 1967, Roger 1981, Woo 1989, Hansen & Knudsen 1992, Sarnari

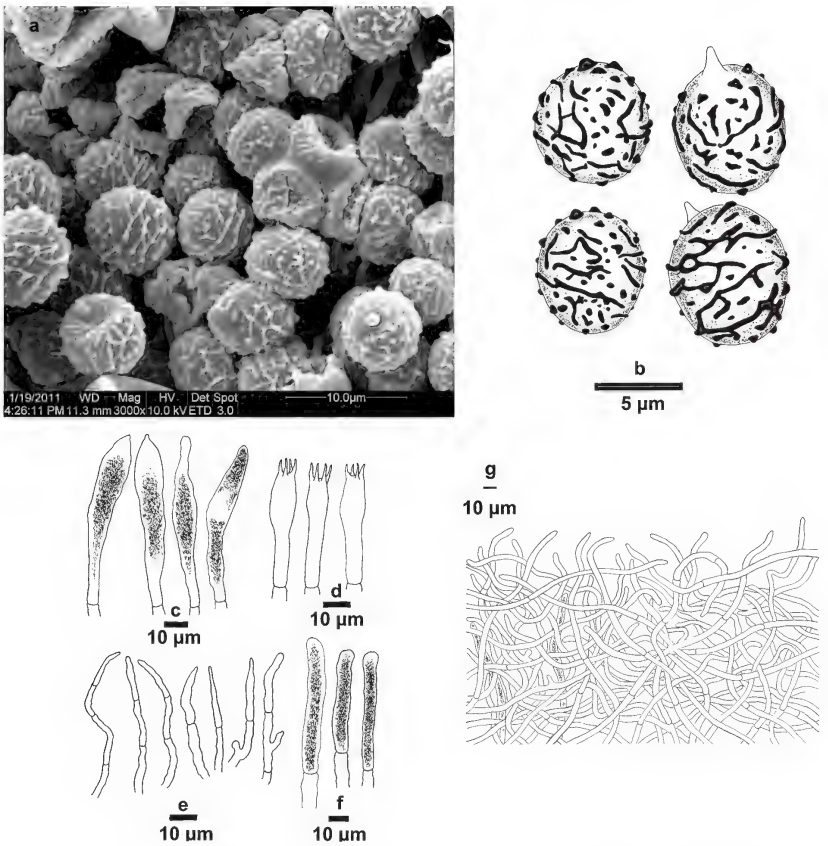

FIG. 2: *Russula atroaeruginea* (HKAS 53626): a. Basidiospores (SEM); b. Basidiospores; c. Pleurocystidia; d. Basidia; e. Hyphal extremities in pileipellis; f. Pileocystidia; g. Pileipellis

BASIDIOSPORES [80/4/3] (6.3–) 6.8–8.1 (–9.0) × (5.9–) 6.1–7.4 (–7.8) μm, Q = (1.01–)1.04–1.23(–1.32) (**Q** =1.15 ± 0.07), subglobose to broadly ellipsoid or (sometimes) ellipsoid, verrucose; ornamentation amyloid, composed of warts mostly fused into fine lines, cristulate to subreticulate, with some isolated verrucae, <1.0 μm high; plage not or indistinctly amyloid. BASIDIA 40–48 × 9–11 μm, clavate, inflated towards upper half, 4–(rarely 2–) spored, projecting 7–13 μm beyond hymenium, hyaline in KOH; sterigmata 3–5 μm, pointed, straight to slightly tortuous. PLEUROCYSTIDIA numerous, 72–115 × 9–13 μm, originating in subhymenium, projecting 35–50 μm beyond hymenium, thin-walled, clavate to subfusiform, sometimes with refractive contents; apices subacute to acute, at times with a moniliform appendage. CHEILOCYSTIDIA not observed; lamellar

1998). *Russula aeruginea* can be distinguished from *R. atroaeruginea* by its more or less discolored pileus, which is often spotted with scattered, rusty spots.

Russula atroaeruginea is also easily distinguished from three other species originally described from France: *R. medullata* grows under deciduous trees of *Corylus*, *Populus*, *Fraxinus*, *Betula*, *Fagus*, *Castanea*, and *Quercus*, has an ochre (III a, b–c) spore print, spores with isolated warts, and rare pileocystidia (Romagnesi 1967, Hansen & Knudsen 1992); *R. stenotricha* has a grayish-green pileus, slightly spicy lamellae, short obtuse pileipellis terminal cells, and short wide pileocystidia 25–50 × 5–9 (12) µm (Romagnesi 1967, Hansen & Knudsen 1992); and *R. anatina* has long cystidia 60–130 × 6.7–12 µm (Romagnesi 1967).

Also similar to *R. atroaeruginea* is the green form of *R. cyanoxantha* (Schaeff.) Fr. (= *R. cyanoxantha* f. *peltereaui*), which differs in its elastic lamellae, white (Ia) spore mass, basidiospores with scattered low warts that are never connected with fine lines, and slender cystidia 65–100 × 5.7–7 µm (Romagnesi 1967, Roger 1981, Hansen & Knudsen 1992).

Among the *Russula* species originally described from China, the morphologically related *R. viridirubrolimbata* J.Z. Ying differs from *R. atroaeruginea* by its finely rimose pileus, unchanging white context, isolated spore ornamentations, hyaline pleurocystidia, and a pileipellis without pileocystidia (Ying 1983).

Russula sichuanensis G.J. Li & H.A. Wen, **sp. nov.** FIGS 3-4

MycoBank MB804645

Differs from *Macowanites yunnanensis* by its larger gastrocarp, dirty white to pale tinged pileus, cream to yellowish lamellae, and larger basidiospores with larger ornamentations.

TYPE: China. Sichuan Province, from Rangtang County to Seda County, banks of Duke River, 5.VIII.2007, Z.W. Ge1707 (**Holotype**, HKAS53792, GenBank JX391969)

ETYMOLOGY: Referring to the type locality region.

BASIDIOMATA semi-hypogeous to epigeous, russuloid to secotioid, small. PILEUS 3.1–4.4 cm in diam., irregularly globose to subglobose when young, hemispheric, barely expanded, sometimes pulvinate when mature, centre slightly depressed with age, whitish, dirty-white to pale-pinkish tinge of Pale Salmon Color (XIV9′f) to Pale Flesh Color (XIV7′f), rarely with greenish tinge of Dark Olive Buff (XL21‴), intermixed with brown to olivaceous brown tinge of Wood Brown (XL17‴), Brussels Brown (III15m) when dry; margin whitish to cream, White (LIII) to Cream color (XVI19′f), often glabrous, dry, slightly viscid when moist, strongly incurved and indented around the stipe, sometimes slightly radially rugose, with slightly pinkish tinge of Pale Flesh Color (XIV7′f), not striate. CONTEXT ≤3 mm at the disc, whitish, fragile; odor not distinctive; taste mild. LAMELLAE/GLEBA 3–6 mm high, sublamellate to lamellate, composed of

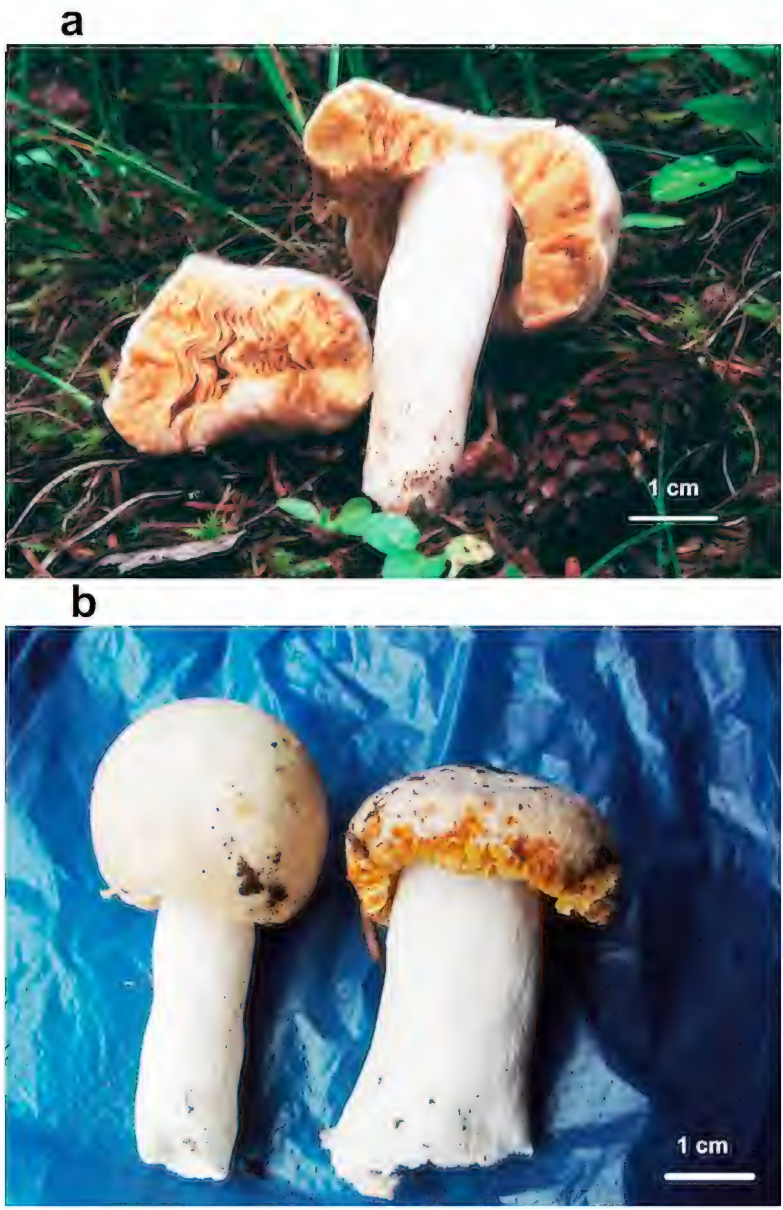

Fig. 3: *Russula sichuanensis*: a. HKAS 45645; b. HKAS 53792 (Holotype)

sinuate, contorted, very crowded, convoluted lamellae, often exposed near the stipe, cream to yellowish tinge of Pale Orange-Yellow (III15f) when juvenile,

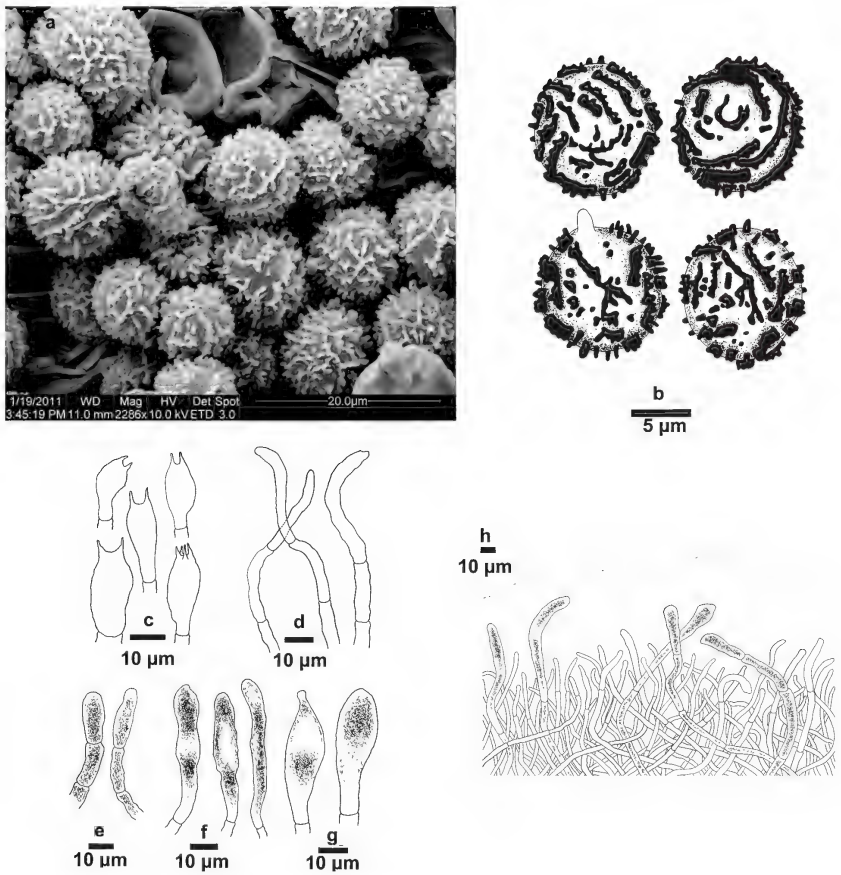

Fig. 4: *Russula sichuanensis* (Holotype HKAS 53792): a. Basidiospores (SEM); b. Basidiospores; c. Basidia; d. Hyphal extremities in pileipellis; e. Pileocystidia; f. Caulocystidia; g. Pleurocystidia.

with pale orange tinge of Orange Buff (III15d) to Light Orange-Yellow (III17d) when mature. Stipe 4.0–6.0 × 1.0–1.5 cm, subcylindrical, surface dry, rugulose longitudinally, dull, without annulus, slightly attenuate upwards, whitish, ≤3 mm at pileus diameter, stuffed first, becoming hollow when old. Spore print ocher to yellow (Romagnesi IIIc–IVa).

Basidiospores [100/3/2] (8.8–)9.4–14.1(–15.5) × (7.6–)7.9–12.8(–13.1) µm, Q = (1.01–)1.02–1.26(–1.32), (**Q** = 1.11 ± 0.08), globose to subglobose, slightly yellowish; ornamentation cristulate to subreticulate, composed of amyloid warts linked as small crests and ridges, forming a partial to nearly complete network, rarely intermixed with isolated verrucae, warts often >1 µm high (but no more than 1.5 µm); suprahilar plage not well defined. Basidia

24–35 × 11–15 µm, mostly with two sterigmata 4–7 µm long, rarely 4–spored, claviform to clavate, short and rather voluminous, rarely cylindrical, hyaline, or sometimes yellow in KOH. Pleurocystidia rare, 45–72 × 10–15 µm, distinctly projecting 10–30 µm beyond the basidia, ventricose, sometimes clavate to sphaeropedunculate, thin-walled, rarely thick-walled, often empty, at times with refringent contents, no reaction in SV. Subhymenium a 20–35 µm thick cellular layer composed of inflated cells 7–13 µm in diam., hyaline, sometimes pale yellowish in KOH. Pileipellis an ixotrichoderm 125–150 µm thick, slightly gelatinized, composed of thin–walled hyphae 3–6 µm wide, cylindrical, hyaline; pileocystidia septate, clavate to cylindrical, 6–10 µm wide, apex sometimes inflated, with refractive contents. Stipitipellis filamentous hyphae 3–6 µm in diam., intermixed with inflated cells, hyaline, some hyphae yellowish to pale ocher in KOH; caulocystidia infrequent, 35–44 × 8–11 µm, cylindrical, septate, with round apex. Clamp connections and laticiferous hyphae absent from all tissues.

Ecology & distribution: Single or gregarious in conifer forests (*Picea* spp.) at altitude of 3300-3900m during July and August, China (Xizang and Sichuan).

Other specimens examined: **China**, **Xizang Autonomous Region**, Jiangda County, Tongpu Township, 31°35′N, 98°23′E, alt. 3300 m, 2.VIII.2004, Z.L. Yang 4266 (HKAS45645); Riwoche County, Sangduo Township, 31°05′N, 96°29′E, alt. 3900 m, 11.VIII.2004, Z.W. Ge335 (HKAS46115); Zuogong County, Wangda Township, alt. 3700 m, 18.VII.2009, Z.L. Yang 5285 (HKAS 57828); **Sichuan Province**, Aba County, Anqiang Township, Ping' an Village, 11.VIII.2007, Z.W. Ge 1800 (HKAS53885, GenBank JX391971).

Comments: Until *Russula* species were emended by Lebel & Tonkin (2007), *Russula sichuanensis* was treated in *Macowanites* Kalchbr., a widespread sequestrate genus containing diverse species phylogenetically close to *Russula* (Miller et al. 2001, Lebel & Castellano 2002, Eberhardt & Verbeken 2004). That very little knowledge is available concerning *Macowanites* in China may result partly from its semi-hypogeous habit; only *M. yunnanensis* M. Zang has been described, based on materials collected from Zixi Mountain, Chuxiong, Yunnan Province (Zang & Yuan 1999). Its morphology conspicuously differs from that of *R. sichuanensis: M. yunnanensis* has a smaller gastrocarp (0.7–1.2 cm broad), a pinkish- to reddish-tinged pileus surface, whitish lamellae, and smaller basidiospores (10–12.5 × 6–7 µm) with 0.3–0.5 µm ornamentations (Zang & Yuan 1999).

Russula sichuanensis resembles a medium-sized, distorted or aborted agaricoid *Russula*, because it has a curly pileus, a white percurrent stipe often covered by the pileus, and tightly contorted sinuate cream to yellow lamellae. Other characters include a cream-tinged pileus (often olivaceous brown at the centre), a yellowish to orange sublamellate gleba, and stipitate basidiomata.

The following microscopical characters are distinctive: the partially reticulate globose to subglobose basidiospores, short voluminous basidia, very rare pleurocystidia, and septate pileocystidia with crystal contents. In addition, the association of *R. sichuanensis* with *Picea* spp. in coniferous forests of the eastern Qinghai–Tibet plateau is a distinctive ecological and biogeographical feature.

A number of species have a similar sublamellate gleba and percurrent stipe: *Macowanites citrinus* Singer & A.H. Sm., *M. chlorinosmus* A.H. Sm. & Trappe, *M. luteolus* A.H. Sm. & Trappe, *M. subolivaceus* A.H. Sm., *Russula galbana* T. Lebel, *R. kermesina* T. Lebel, *R. luteirosea* (Bougher) T. Lebel, and *M. arenicola* S.L. Mill. & D. Mitch. However, *M. citrinus*, originally described from Idaho (in the northwestern U.S.A.), differs from *R. sichuanensis* in having smaller subglobose to short ellipsoid basidiospores ornamented by conical spines and short flattened ridges and with a small indistinct suprahilar plage (Pegler & Young 1979), and *M. chlorinosmus* (from Oregon, U.S.A.) has a rimose pileus cutis, a strong chlorine odor, a very unpleasant taste, and basidiospores with low warts (Smith 1963). *Macowanites luteolus* (also from Oregon) differs by its short thin stipe, smaller basidiospores with scattered to close 0.3–0.7 μm ornamentations, subhymenium cells that are hyaline in KOH, and numerous macrocystidia (Smith 1963). *Macowanites subolivaceus* (from Idaho) has a lobed to very irregular pileus margin, a very fragile thin context, basidiospores 7–9 μm in diam., and a peridial epicutis consisting of a turf of gelatinous branched hyphae with 60–200 μm long pseudocystidia (Smith 1963). *Russula galbana* (from Queensland, northeastern Australia) has smaller basidiomata, a short slender stipe, and common, dimorphic hymenophoral cystidia with obtuse or mucronate apices (Lebel & Tonkin 2007); *R. kermesina*, (from Nelson, New Zealand) is characterized by its carmine red to dark-red-tinged pileus, chalk-white gleba, large cystidia not or only slightly projecting beyond the basidia, and pileipellis without pileocystidia (Label & Castellano 2002). *Russula luteirosa* (from western Australia) has a pileus with intermixed cream to pale yellowish and rosaceous colors, large basidia, and an association with *Allocasuarina*, *Acacia*, and *Eucalyptus* (Bougher 1997). *Macowanites arenicola* (from northwestern Florida, U.S.A.) has a yogurt- or soy sauce-type odor, smaller broadly elliptical to elliptical spores with low ornamentation (8.8–10.4 × 6.8–7.2 μm, Q = 1.37, verrucae 0.2–0.4 μm high), long slender cystidia (80–120 × 6–7.5 μm), and grows in white quartzite sands of costal scrub forests (Miller 2004).

Russula sichuanensis also shows some superficial affinities with *Russula* sect. *Laricinae* Romagn. for its small-sized basidiocarps, ocher to yellow spore print, short basidia, rare pleurocystidia, and pluriseptate dermatocystidia (Romagnesi 1985), but its russuloid to secotioid basidiocarps and large basidiospores obviously distinguish it from the agaricoid sect. *Laricinae*.

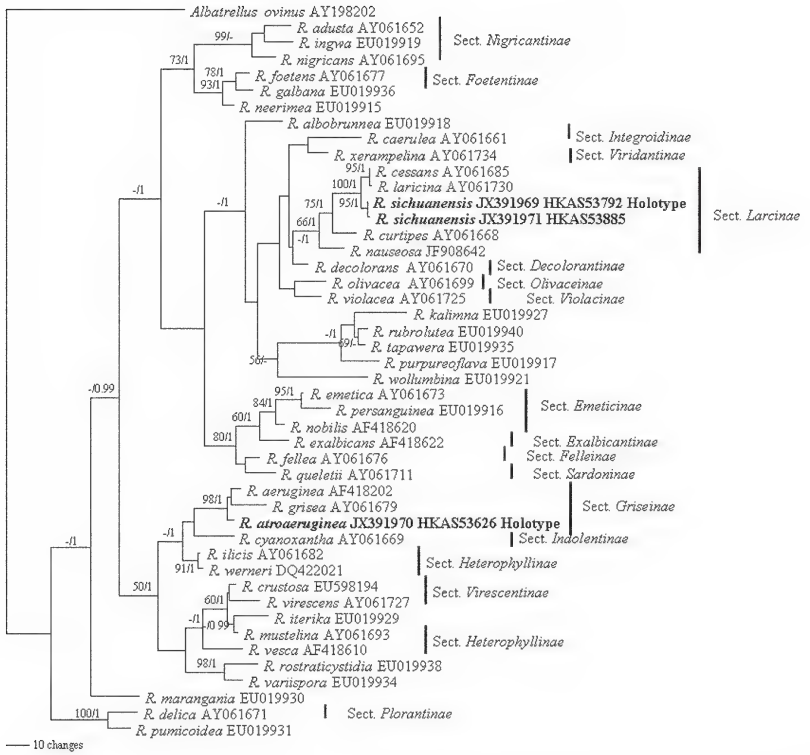

FIG. 5. Phylogenetic relationship tree of *Russula* species inferred from the dataset of ITS1-5.8S-ITS2 sequences. Consistency index (CI) = 0.461, retention index (RI) = 0.665, and tree length (TL) = 1030. MP bootstrap support values (>50%) and PP (>0.94) from Bayesian Analysis are indicated above or below the branches as MP BS/BA PP. Sectional names are from Romagnesi (1987).

Phylogenetic results

The ITS1-5.8S-ITS2 dataset included sequences representing 44 taxa representing nine *Russula* subgenera. The aligned sequence comprised 471 nucleotide sites. In the MP analysis, 195 characters were constant, 69 variable characters were parsimony-informative, and 207 characters were parsimony-uninformative. In the ITS phylogenetic tree (FIG. 5), *R. atroaeruginea* clustered with *R. aeruginea* and *R. grisea* with strong statistical support (98% boot strap, 1.0 posterior probability). A Blast comparison of *R. atroaeruginea* (JX391970) and *R. aeruginea* (AF418612) through the NCBI databank shows a 93% max identification and 96% coverage. Another ITS blast with *R. atroaeruginea* (JX391970) and *R. grisea* (AY061679) shows a 90% max identification of 99% coverage, confirming that *R. atroaeruginea* is related to *R. grisea* / *aeruginea* group in *Russula* sect. *Griseinae*. The separation between *R. atroaeruginea* and

R. cyanoxantha is also well supported. These phylogenetic results suggest that *R. atroaeruginea* and *R. aeruginea* are closely related but different species.

The clade in which *R. sichuanensis* is located is highly supported (Fig. 5). Members of *Russula* subgen. *Tenellula* sect. *Larcinae* cluster together, suggesting that *R. sichuanensis* is closely related to the *R. laricina* / *cessans* group. However, given the lack of stability in the topologies due to GenBank's limited number of ITS sequences from closely related secotioid *Russula* taxa, we hesitate making a definitive statement about the phylogenetic position of *R. sichuanensis*. Broader collections from East Asia, particularly of russuloid to secotioid *Russula* taxa, are required in future work.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 30770013), International Cooperation Program of Ministry of Science and Technology of the People's Republic of China (No. 2009DFA31160) and the Hundred Talents Program of the Chinese Academy of Science. The authors are very grateful to Dr. Y.C. Dai (Institute of Applied Ecology, Chinese Academy of Sciences) and Dr. J.P. Xu (Department of Biology, McMaster University) for reviewing the manuscript; Dr. Z.W. Ge for assistance in specimen collecting; Dr. L.S. Wang, Dr. E.D. Liu, and Dr. H. Peng (Kunming Institute of Botany, Chinese Academy of Sciences) for the loan of herbarium specimens; Mr. S. Shi (Institute of Botany, Chinese Academy of Sciences) for providing references; Ms. X.F. Zhu for inking in line drawings; to Dr. X.L. Wang (Institute of Microbiology, Chinese Academy of Sciences) for providing advice and suggestions regarding phylogenetic analysis; Dr. B.A. Jaffee (University of California Davis), Dr. Z.L. Yang (Kunming Institute of Botany, Chinese Academy of Sciences), Dr. J.Y. Zhuang (Institute of Microbiology, Chinese Academy of Sciences), Dr. S.R. Pennycook (Landcare Research) and Dr. L.L. Norvell (Pacific Northwest Mycology Service) for improving the manuscript; and Dr. C.L. Li and Ms. J.N. Liang (Institute of Microbiology, Chinese Academy of Sciences) for providing assistance in SEM photography.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.189

Volume 124, pp. 189–208 *April–June 2013*

Five new *Terfezia* species from the Iberian Peninsula

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Abstract — A phylogenetic analysis of Iberian *Terfezia* collections indicates eight clades, of which three correspond with previously described species (*T. alsheikhii*, *T. fanfani*, *T. olbiensis*) and five are described as new species — *T. albida*, *T. eliocrocae*, *T. extremadurensis*, *T. pini*, and *T. pseudoleptoderma*. These results are supported by the morphology of the examined *Terfezia* ascomata that were identified by their ecological patterns and chorology, as well as by phylogenetic analyses. Morphological features and ITS-rDNA sequence analyses from identified *Terfezia* specimens are described. A key to these *Terfezia* species is also provided.

Key words — desert truffle, hypogeous, mycorrhizal fungi, *Pezizaceae*

Introduction

The genus *Terfezia* (Tul. & C.Tul.) Tul. & C.Tul. includes twelve species (Kirk et al. 2008) of hypogeous ascomycetes formerly placed in the family *Terfeziaceae* within the order *Pezizales* (Trappe 1979). However, based on molecular and morphological characters, *Terfezia* is now accepted in the *Pezizaceae* (Norman & Egger 1999; Percudani et al. 1999; Laessøe & Hansen 2007). These hypogeous fungi are edible and are known as "desert truffles" due to their habitat in typically arid and semi-arid ecosystems, mostly in the Mediterranean region (Morte et al. 2009).

Most *Terfezia* species establish mycorrhizal symbiosis with plants from family *Cistaceae*, mainly with perennial and annual *Helianthemum* species (Dexheimer et al. 1985; Fortas & Chevalier 1992; Gutiérrez et al. 2003) or with trees from different phyla (Díez et al. 2002; Taylor et al. 1995). These plants and their associated fungi play a major role in maintaining Mediterranean shrub lands and xerophytic grasslands and thus in preventing erosion and

desertification (Honrubia et al. 1992). This mycorrhizal association is well adapted to semiarid climates through the physiological mechanism of drought avoidance (Morte et al. 2000, 2010).

Six *Terfezia* species have been reported so far from the Iberian Peninsula: *T. claveryi* Chatin, *T. boudieri* Chatin, *T. arenaria* (Moris) Trappe, *T. leptoderma* Tul. & C. Tul., *T. olbiensis,* and *T. alsheikhii*. *Terfezia claveryi*, *T. boudieri,* and *T. olbiensis* have been recorded from basic soils and *T. arenaria* from acid soils, but all four in mycorrhizal association with different *Helianthemum* species. However, *T. leptoderma* has been found in association with *Tuberaria guttata* L. Fourr. in acid soils, with *Cistus* in slate-derived soils, and with *Quercus ilex* L. and *Pinus halepensis* Mill. in basic soils (Díez et al. 2002). Isolations from *Cistus*, *Pinus*, and *Quercus* could belong to distinct species with different host or/and edaphic specialization. These authors emphasized that the sampling in these ecosystems is scanty and further study is necessary. Moreover, the small spores of *T. leptoderma* specimens collected under pine and evergreen holm oak fit those of *T. olbiensis,* which was described as morphologically similar to *T. leptoderma*, except for slightly smaller spores and shorter spines (Díez et al. 2002). There is a certain consensus that *T. olbiensis* is an immature form and a synonym of *T. leptoderma* (Díez et al. 2002; Moreno et al. 1986).

The main objective of this study was to describe five new *Terfezia* species. For this purpose, we conducted classical morphological studies complemented by phylogenetic analyses based on ITS-rDNA sequences from *Terfezia* specimens collected throughout the Iberian Peninsula.

Materials & methods

Fungal specimens

Ascomata of *Terfezia* spp. were collected in different years and from different locations in the Iberian Peninsula (Spain and Portugal). The ascomata were collected from cracks in the soil close to host plants. Characterized and sequenced specimens are listed in Table 1. Generally, three specimens per collection were used when the material was fresh. Furthermore, conserved dried material was processed. Soil characters were obtained from the Spanish Geological Map, according to FAO-CEE, 1998 from the "Atlas Nacional de España" (IGN). The different host plants were identified using Flora Ibérica keys (Muñoz-Garmendia & Navarro 1993).

External ascocarp characteristics (shape, colour, appearance) were recorded in detail. Ascomata were then cut and the morphology of the peridium and gleba was described. Asci and ascospores were examined using an Olympus BX51 microscope equipped with a digital camera (Canon PSpro1). A minimum of 100 asci, ascospores, and peridial warts was measured per species. The ascospores of *Terfezia eliocrocae* and *T. pseudoleptoderma* were stained with acid fuchsine solution (0.01% acid fuchsine in acetic acid, ethylene glycol, and lactic acid, 1:1:1, v/v/v) to improve visualization.

Samples of the collected specimens are stored in the Mycological Herbarium of the Botany Area of the University of Murcia (MUB).

TABLE 1. *Terfezia* collections analyzed

CLADE	COLLECTION* #	ORIGIN ^ & HOST	YEAR, COLLECTOR	GENBANK Nº
1	tO9*	Belvis (Cc). *Tuberaria guttata*	2009. J. Mohedano	HM056199
1	tO10*	Belvis (Cc). *T. guttata*	2009. J. Mohedano	HM056200
1	tO11*	Belvis (Cc). *T. guttata*	2009. J. Mohedano	HM056201
1	tO17*	Valdecañas (Cc). *T. guttata*	2009. J. Mohedano	HM056202
1	tO18	Valdecañas (Cc). *T. guttata*	2009. J. Mohedano	
1	tO19	Valdecañas (Cc). *T. guttata*	2009. J. Mohedano	
1	tO20*	Castuera (Ba). *T. guttata*	2009. M. Romero	HM056203
1	tO21	Castuera (Ba). *T. guttata*	2009. M. Romero	
1	tL9	Valdecañas (Cc). *T. guttata*	2007. J. Mohedano	
1	tL10*	Valdecañas (Cc). *T. guttata*	2007. J. Mohedano	HM056204
1	tL11	Valdecañas (Cc). *T. guttata*	2007. J. Mohedano	
1	tL12	Valdecañas (Cc). *T. guttata*	2007. J. Mohedano	
1	#*	C. Arañuelo (Cc). *T. guttata*	2002. J. Díez	AF276678
2	tO4*	Lorca (Mu). *Helianthemum*	2001. A. Morte	HM056205
2	tO7*	Lorca (Mu). *Helianthemum*	2007. A. Morte	HM056206
3	tTP1	Montesihno. Portugal. *Cistaceae*	2006. A. Rodríguez	
3	tTP2*	Montesihno. Portugal. *Cistaceae*	2006. A. Rodríguez	HM056207
3	tL13	Quintana (Ba). *Cistaceae*	2008. M. Romero	HM056208
3	#*		2011. G. Kovacs	HQ698098
3	#		2011. G. Kovacs	HQ698100
4	tO31	Sieteiglesias (Va). *Quercus*	2003. A. García	
4	tO32*	Ronquines (Va). *Pinus*	1998. A. García	HM056209
4	tO33	Ronquines (Va). *Pinus*	1998. A. García	
4	tO35	Cornudilla (Bu). *Pinus*	2008. F. Sainz	
4	tO36	Cornudilla (Bu). *Pinus*	2008. F. Sainz	
4	tO37	Cornudilla (Bu). *Pinus*	2008. F. Sainz	
4	tO40*	Cornudilla (Bu). *Pinus*	2008. F. Sainz	HM056210
4	#*	Madrid. *Pinus*	2008. Barriuso et al.	DQ386140
5	tL16*	Villafranca (Bu). *Cistaceae*	2008. F. Sainz	HM056211
5	tL17*	Villafranca (Bu). *Cistaceae*	2008. F. Sainz	HM056212
5	tL18	Villafranca (Bu). *Cistaceae*	2008. F. Sainz	
5	tO42*	Valdecañas (Cc). *Pinus*	2010. J. Mohedano	HM056213
5	#*	Madrid	2008. A. Rincón	FJ013064
6	tL1*	Belvis (Cc). *T. guttata*	2009. J. Mohedano	HM056214
6	tL2	Belvis (Cc). *T. guttata*	2009. J. Mohedano	
6	tL3	Belvis (Cc). *T. guttata*	2009. J. Mohedano	
—	tO22*	Castuera (Ba). *Cistaceae*	2009. M. Romero	HM056215
6	tO23*	Garrovilla (Cc). *T. guttata*	2004. A. Mateos	HM056216
6	tO24	Garrovilla (Cc). *T. guttata*	2004. A. Mateos	
6	tO30	Garrovilla (Cc). *T. guttata*	2004. A. Mateos	
6	tL6	Valdecañas (Cc). *T. guttata*	2001. J. Mohedano	
6	tL7*	Valdecañas (Cc). *T. guttata*	2001. J. Mohedano	HM056217
6	tL8*	Cortas Blas (Va). *Cistaceae*	1998. A. García	HM056218
6	tL14*	Quintana (Ba). *T. guttata*	2009. M. Romero	HM056219
6	tL15	Quintana (Ba). *T. guttata*	2009. M. Romero	
6	#*	Cañaveral (Hu). *Cistaceae*	2002. J. Díez	AF396862
6	#*	—	2002. J. Díez	AF276676

Table 1, concluded

Clade	Collection* #	Origin ^ & Host	Year, Collector	GenBank nº
6	#*	Madrid.	2008. A. Rincón	FJ013087
—	#*	Valencia. *Pinus*	2002. J. Díez	AF396864
7	tO15*	Ballestero (Ab). *Helianthemum*	2009. A. Rodríguez	HM056220
7	tO16*	Albacete (Ab). *Helianthemum*	2009. A. Rodríguez	HM056221
7	#*	Murcia. *Helianthemum*	2001. A. Gutiérrez	AF387655
8	tO12*	Masegoso (Ab). *Quercus*	2009. A. Rodríguez	HM056222
8	tO13	Masegoso (Ab). *Quercus*	2009. A. Rodríguez	
8	tO14*	Masegoso (Ab). *Quercus*	2009. A. Rodríguez	HM056223
8	tO34*	Santa Espina (Va). *Pinus*	1998. A. García	HM056224
8	tO39*	Onteniente (V). *Pinus*	2009. F. García	HM056225
8	#*	Murcia. *Pinus*	2001. Gutiérrez et al	AF387656
8	#*	—	2002. J. Díez	AF276677
8	#*	France. *Quercus*	2002. J. Díez	AF396863
—	#*	Jarada. Morocco	1994. M. Achouri	AF301421
—	#*	C. Arañuelo (Cc).	2002. J. Díez	AF276674
—	#*	Zeelim. Israel	1996. Bedouins	AF092098

* = included in the cladistic analysis; # = external sequence.

^ Spanish provinces: Ab: Albacete; Ba: Badajoz; Bu: Burgos; Cc: Cácerese; Hu: Huelva; Mu: Murcia; V: Valencia; Va: Valladolid.

Macroscopic and microscopic characterization

For identification, ascomata were compared with descriptions from Mattirolo (1900, 1906, 1907, 1922), Lázaro Ibiza (1908), Malençon (1938, 1973), Ceruti (1960), Cerutti et al. (2003) and keyed according to Montecchi & Lazzari (1993) and Montecchi & Sarasini (2000). Trappe (1971, 1979), Calonge et al. (1977) and Honrubia et al. (1992) were also taken into account. The descriptions of *Tuber lutescens* Lázaro Ibiza (≡ *Terfezia lutescens* (Lázaro Ibiza) Malençon) and *T. pallidum* Lázaro Ibiza (≡ *T. pallida* (Lázaro Ibiza) Malençon) were also consulted (Lázaro Ibiza 1908, Malençon 1938). In addition, descriptions of *T. leptoderma*, *T. fanfani*, *T. cadevalli* Font Quer, *T. hafizi* Chatin, *T. berberiodora* Lesp. ex Tul. & C.Tul., and *T. goffartii* Chatin were also checked.

DNA analyses

Genomic DNA was isolated from 150–200 mg of the inner gleba of the ascocarps using the E.Z.N.A. Fungal DNA kit (Omega Bio-Tek, Doraville, GA, USA) and following the manufacturer's instructions. The Internal Transcribed Spacer (ITS) region of the rDNA, including the 5.8S ribosomal gene, was amplified using the universal ITS5 and ITS4 primers (White et al. 1990). All PCR amplifications were carried out in a final volume of 25 µL containing 0.2 mM of each dNTP, 0.6 µM of each primer, 3.75 mM $MgCl_2$, 1X PCR buffer and 1.25 U of Taq DNA polymerase (Invitrogen, California, USA).

PCR reactions were performed in a Mastercycler Gradient thermocycler (Eppendorf, Hamburg, Germany) with the following cycling parameters: an initial denaturalization step for 2 min at 94°C, 30 cycles consisting of 30 s at 94°C, 1 min at 65°C, and 1 min at 72°C, and a final extension at 72°C for 4 min.

PCR products were purified using the E.Z.N.A. Cycle-Pure kit (Omega Bio-Tek) following the manufacturer's instructions. Clean PCR products were sequenced in both directions at the Molecular Biology Service (University of Murcia). The BioEdit (Hall

1999) program was used to obtain the consensus sequence of each sample. Sequences were deposited on GenBank (NCBI) under accession numbers indicated in Table 1.

Phylogenetic analyses

Nucleotide Basic Local Alignment Search Tool (BLAST) searches (megablast) were used to compare the sequences obtained in this study with other DNA sequences in GenBank (NCBI, Altschul et al. 1997) to provisionally identify the specimens prior to phylogenetic analyses. The sequence alignment was carried out with ClustalW (MEGA software, version 4; Tamura et al. 2007), following the default options. A minimal manual adjustment was made with the MEGA v.4 editor. Alignments are available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S10906?x-access-code=eadd311aaa59cd96424d699af26cb699&format=html)

Evolutionary history was inferred based on the Maximum Parsimony (MP; Eck & Dayhoff 1966) and the Minimum Evolution (ME; Rzhetsky & Nei 1992) methods using MEGA v.4. The closest species not belonging to the sister group were selected as outgroups. The bootstrap consensus tree inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed. The branches corresponding to the partitions reproduced in less than 50% bootstrap replicates were excluded. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985).

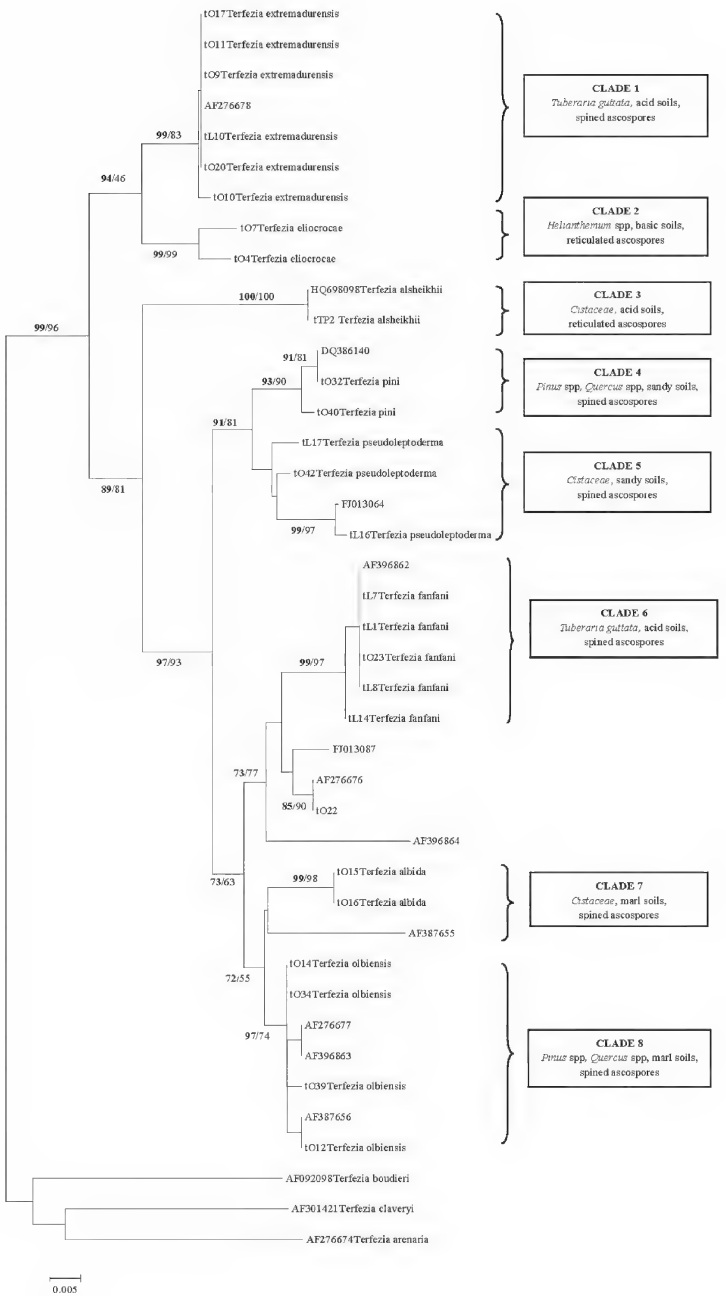
The MP tree (Plate 1) was obtained using the Close-Neighbour-Interchange algorithm at search level 3 with which the initial trees were obtained with the random addition of sequences (10 replicates). The tree was drawn to scale, and branch lengths were calculated using the average pathway method (Nei & Kumar 2000). The number of changes over the whole sequence is presented in units. All positions with gaps and missing data were eliminated from the dataset (the Complete Deletion option). The final dataset contained a total of 429 positions, of which 69 were parsimony informative.

The ME tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are presented in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbour-Interchange (CNI) algorithm (Nei & Kumar 2000) at search level 1. The Neighbour-joining algorithm (Saitou & Nei 1987) was used to generate the initial tree. All the positions with gaps and missing data were eliminated from the dataset (the Complete Deletion option). The final dataset contained a total of 429 positions.

The sequences from *T. claveryi, T. boudieri* and *T. arenaria* were chosen as outgroup.

Phylogenetic results

Sequence analyses of the ITS-rDNA from the examined samples produced two trees based on the Maximal Parsimony (MP) and the Minimum Evolution (ME) methods, both with a virtual sampling or bootstrap of 1000 replicas. In the ME phylogeny, the 38 sequences are clearly distributed in eight well-separated lines (Plate 1) that match the eight phenetically described species.


tO17Terfezia extremadurensis
tO11Terfezia extremadurensis
tO9Terfezia extremadurensis
AF276678
tL10Terfezia extremadurensis
tO20Terfezia extremadurensis
tO10Terfezia extremadurensis
tO7Terfezia eliocrocae
tO4Terfezia eliocrocae
HQ698098Terfezia alsheikhii
tTP2 Terfezia alsheikhii
DQ386140
tO32Terfezia pini
tO40Terfezia pini
tL17Terfezia pseudoleptoderma
tO42Terfezia pseudoleptoderma
FJ013064
tL16Terfezia pseudoleptoderma
AF396862
tL7Terfezia fanfani
tL1Terfezia fanfani
tO23Terfezia fanfani
tL8Terfezia fanfani
tL14Terfezia fanfani
FJ013087
AF276676
tO22
AF396864
tO15Terfezia albida
tO16Terfezia albida
AF387655
tO14Terfezia olbiensis
tO34Terfezia olbiensis
AF276677
AF396863
tO39Terfezia olbiensis
AF387656
tO12Terfezia olbiensis
AF092098Terfezia boudieri
AF301421Terfezia claveryi
AF276674Terfezia arenaria
0.005
99/83
94/46
99/99
100/100
99/96
91/81
93/90
91/81
89/81
99/97
97/93
99/97
73/77
85/90
73/63
99/98
72/55
97/74
CLADE 1
Tuberaria guttata, acid soils, spined ascospores
CLADE 2
Helianthemum spp, basic soils, reticulated ascospores
CLADE 3
Cistaceae, acid soils, reticulated ascospores
CLADE 4
Pinus spp, Quercus spp, sandy soils, spined ascospores
CLADE 5
Cistaceae, sandy soils, spined ascospores
CLADE 6
Tuberaria guttata, acid soils, spined ascospores
CLADE 7
Cistaceae, marl soils, spined ascospores
CLADE 8
Pinus spp, Quercus spp, marl soils, spined ascospores

The MP phylogenetic topology is consistent with that of the ME analysis in which the same nodes and clusters are reproduced (Plate 1). The cladogram distinguishes eight clades (Table 2).

Table 2. Diagnostic characters of clades and *Terfezia* spp.

Clade	Species	Bootstrap %	Soil	Host
1	*T. extremadurensis*	94	Acid	*Tuberaria guttata*
2	*T. eliocrocae*	94	Alkaline	*Helianthemum* spp.
3	*T. alsheikhii*	89	Acid	*Cistaceae*
4	*T. pini*	91	Acid	*Pinus* spp., *Quercus* spp.
5	*T. pseudoleptoderma*	91	Acid	*Cistaceae*
6	*T. fanfani*	70	Acid	*Tuberaria guttata*
7	*T. albida*	70	Alkaline	*Helianthemum* spp.
8	*T. olbiensis*	70	Alkaline	*Pinus* spp., *Quercus* spp.

Taxonomy

The morphology and distributions of the examined specimens support eight phenetic species, described below (presented in clade order; Plate 1).

Terfezia extremadurensis Muñoz-Mohedano, Ant. Rodr. & Bordallo, **sp. nov.**

MycoBank MB561591 Plate 2a,b

Differs from all other spiny-spored *Terfezia* spp. by its larger spores with wider spines and its *Tuber*-like glebal morphology.

Type: Spain, Extremadura, Cáceres, Belvis de Monroy, 17 March 2009, leg Muñoz-Mohedano (**Holotype**, MUB Fung-0026).

Etymology: referring to Extremadura, the western Spanish region where the species was first identified

Ascomata hypogeous to partially emergent at maturity, 2–5 cm, subglobose, sometimes furrowed and nodulose, often cracked, often with a small basal depression, rarely with pseudostipe, cream colour at first, becoming brown, black spots on the sun-exposed parts or where handled, smooth. Peridium 300–600 µm thick, well-defined, concolorous with surface in cross section, pseudoparenchymatous, composed of subglobose cells, hyalines and thin-walled in the innermost layers, yellowish and with thicker walls in the outermost layers. Gleba solid, fleshy, succulent, whitish at first, soon becoming salmon pink, darkening with age, greenish grey at maturity, marbled with thin, white,

Plate 1. Minimum Evolution (ME) and Maximum Parsimony (MP) consensus tree showing evolutionary relationships of 38 sequences derived from the ITS-rDNA region. On each branch, % of 1000 bootstrapping replicates supported by ME is shown first (in bold) and by MP second (in plain font). Host plant, soil, and ascospore information is presented for each clade. Non-significant bootstrap values under 70 are not included.

meandering veins, sometimes arising from the base and inconspicuous in very mature specimens; frequently with small holes indicating mycophagous activity. Odour faint, not distinctive. Asci inamyloid, subglobose to ovate, sessile or short-stipitate, 60–80 × 50–65 µm, walls 1–2 µm thick, with 6–8 irregularly disposed spores, randomly arranged in the gleba. Ascospores globose, (21–) 22–26(–27) µm diam (median = 24 µm) including ornament [(16–)17–19(–20) µm (median = 18 µm) without ornament], hyaline, smooth and uniguttulate at first, by maturity yellow and ornamented with conical, blunt, thick spines, sometimes truncated, sometimes finger-like, often joined at the base, 3–4(–5) µm long, 1–3 µm wide at the base.

Ecology & distribution — Widely distributed in the western half of the Iberian Peninsula, common in grassland of Extremadura in sandy, acid soils, associated exclusively with *Tuberaria guttata*, from late winter to early spring. This is the earliest *Terfezia* species to appear (in March) associated with *T. guttata*.

Additional collections examined: **SPAIN**: **Extremadura**, Cáceres: Valdecañas, 2007, Muñoz-Mohedano (MUB Fung-0012); 2009, Muñoz-Mohedano (MUB Fung-0035); Badajoz: Castuera, 2009, M. Romero (MUB Fung-0036).

Comments — *Terfezia extremadurensis* differs from other spiny-spored *Terfezia* species in its *Tuber*-like gleba, with meandering veins of the gleba not completely surrounding the fertile tissue and not forming pockets as is typical for all other *Terfezia* species. *Terfezia fanfani*, described below, shares the same habitat but has reddish tones, a different gleba, and smaller spores (19–22 µm) with thin spines 1 µm wide at the base.

Clade 1 comprises *T. extremadurensis* and clearly differs from the other *Terfezia* species in the shape of the gleba (reminiscent of *Tuber*) and the ascospores with truncated crested spines. As far as we are aware, these two characteristics have not been described to date for *Terfezia.* The phylogenetic analyses clearly support with a high bootstrap value this taxon in an independent clade (Plate 1). Despite the similarity of *T. lutescens* (Malençon 1938) to *T. extremadurensis*, the absence of any description of spines with crossed crests (an undoubtedly visible character) by Lázaro Ibiza (1908) leads us to think that it is actually a different species. However, we were unable to find the original samples of Lázaro Ibiza to compare their ITS-rDNA sequences.

Terfezia eliocrocae Bordallo, Morte & Honrubia, **sp. nov.** Plate 2c,d

MycoBank MB561592

Differs from other reticulated spored *Terfezia* spp by its friable and crumbly gleba that remains milky white when exposed.

Type: Spain, Murcia, Lorca, Zarzadilla de Totana, 21 April 2007, leg A. Morte (**Holotype**, MUB Fung-0015).

Etymology: referring to Eliocroca, old Roman name for the city of Lorca (Murcia, Spain), and its surrounding area, where the type specimen was collected.

Ascomata more or less crushed shapes, roughened, embossed, >5 cm. Cream colour, blackish aspect when ripe with a rough, cracked texture. Sticky mycelial remains and pseudo-stipe. Peridium thin, well delimited, inseparable from the gleba, cream to blackish-brown colour, partially rough when ripe; pseudoparenchymatous, ≤200 µm thick, cells round, 30 × 15 µm; external layer with hyphal extensions. Gleba intensely white, remaining white and not darkening when mature or in contact with air; both fertile and sterile tissues milky white with small islets of fertile tissue surrounded by very thin ramified sterile veins. Asci 8-spored, subglobose, ovoid or pyriform elongated. Ascospores spherical with a well-developed reticulum, regular, 17–19 µm diam. including ornamentation; at first hyaline and smooth, in age becoming yellow with a very marked (≤1 µm tall) reticulum.

Habitat — Growing in grasslands on calcareous soils with *Helianthemum almeriense* and *H. violaceum* (*Cistaceae*).

Additional collections examined: **SPAIN**: **Murcia**: Totana, 2001, A. Morte (MUB Fung-0005); 2007, A. Morte (MUB Fung-0016).

Comments — *Terfezia eliocrocae* (Clade 2), distinguished by inner milky white colour that persists in contact with air, differs from *T. berberiodora* (characterized by hazelnut-shaped ascocarps, an atypical gleba in islets, and a characteristic flavour of *Berberis vulgaris*) in its smaller spores and larger ascocarps (Castro & Freire 1982). *Terfezia hafizi* also produces slightly reticulate but larger (18–20 µm) ascospores (Chatin 1892). The markedly reticulate ascospores distinguish *T. eliocrocae* from *T. hafizi* and *T. alsheikhii* (see below).

Terfezia alsheikhii Kovács, M.P. Martín & Calonge, Mycologia 103: 848. 2011.

Plate 2e,f

Ascomata regular, globose, 0.5–1.5 cm, smooth, firm; no strong odour detected. Peridium smooth, thin, inseparable from the gleba; brown-ochre; pseudoparenchymatous throughout, with thick-walled cells ≤50 µm in diameter. Gleba formed by big pinkish islets of fertile tissue, varying in size and surrounded by a scarce sterile white tissue, stained by brown-reddish zones when in contact with air. Asci primarily 8-spored, subglobose, ovoid, pyriform, 60–100 × 40–70 µm, sessile or with a short thick peduncle. Ascospores spherical, reticulate, 15–18 µm diam. (including ornamentation); at first hyaline, yellow when mature; decorated by an irregular, well-developed reticulum with thickish (nearly 1 µm) net; very mature spores with roundish to flat warts ≤2 µm × 2 µm.

Habitat — *Terfezia alsheikhii* grows in sandy acidic soils associated with cistaceous plants.

Collections examined: **SPAIN**: **Extremadura**, Badajoz: Castuera, 2009, M. Romero (MUB Fung-0034). **PORTUGAL**: Braganza: Portelo, Montesinho Natural Park, 2006, A. Rodríguez (MUB Fung-0009).

Comments — Gregarious specimens have been found by raking under *Cistus monspeliensis* L. on the border of a forest track.

Terfezia pini Bordallo, Ant. Rodr. & Muñoz-Mohedano, **sp. nov.** Plate 2G,H

MycoBank MB561594

Differs from *T. fanfani* and *T. pseudoleptoderma* by its spores with long spines joined at their bases to form a pseudo-reticulum.

Type: Spain, Burgos, Cornudilla, 8 March 2008, leg F. Sáinz (**Holotype**, MUB Fung-0027).

Etymology: referring to pinewoods, its preferred habitat.

Ascomata globose, round, regular, <2 cm diam., smooth, base often with residual mycelium. Peridium smooth, slightly tomentose, thin, not clearly delimited and difficult to separate from the gleba, 200–400 µm thick; at first cream colour, becoming ochre, grey in cross-section grey, partially covered by a whitish film; pseudoparenchymatous, cells thick-walled, ≤40 µm diam., pigmented in the outer part. Gleba initially whitish; when mature, the fertile tissue forming round islets of various sizes, initially pale pinkish becoming greenish brown and greyish when very mature and always surrounded by a sterile tissue of white veins. Asci 6–8-spored, ovoid, ellipsoid, subglobose, sessile, 60–90 × 45–60 µm, walls 1µm thick. Ascospores spherical with spines, 20–23(–25) µm (including ornamentation); initially hyaline and smooth with a big central drop, when mature, yellowish-ochre and decorated by cylindrical spines (3–4(–5) × 1 µm) with a round tip, joined basally to form crests, often in a pseudo-reticulum.

Ecology — The species grows mainly in sandy pine forests from November to May. It is rarely observed on the surface and is often found at a depth of 3–5 cm, usually under mosses.

Additional collections examined: **SPAIN**: **Castile & León**, Valladolid: Sieteiglesias, 2003, Aurelio García (MUB Fung-0007); Ronquines, 1998, Aurelio García (MUB Fung-0010).

Comments — *Terfezia pini* (represented by Clade 4) is diagnosed by its occurrence on burnt areas under pine and oak and lack of association with cistaceous plants and its spore ornamentation comprising long spines joined at the bases to form a pseudo-reticulum. A high bootstrap value in the phylogenetic analyses strongly supports its separation from other species (Plate 1).

The spine crests of *T. pini* resemble the lamelliform folds of the non-reticulate episporium of *T. cadevalli* (Solá 1925), which is distinguished in that it forms neither a reticulum nor clearly identifiable spines). The larger ascospores of

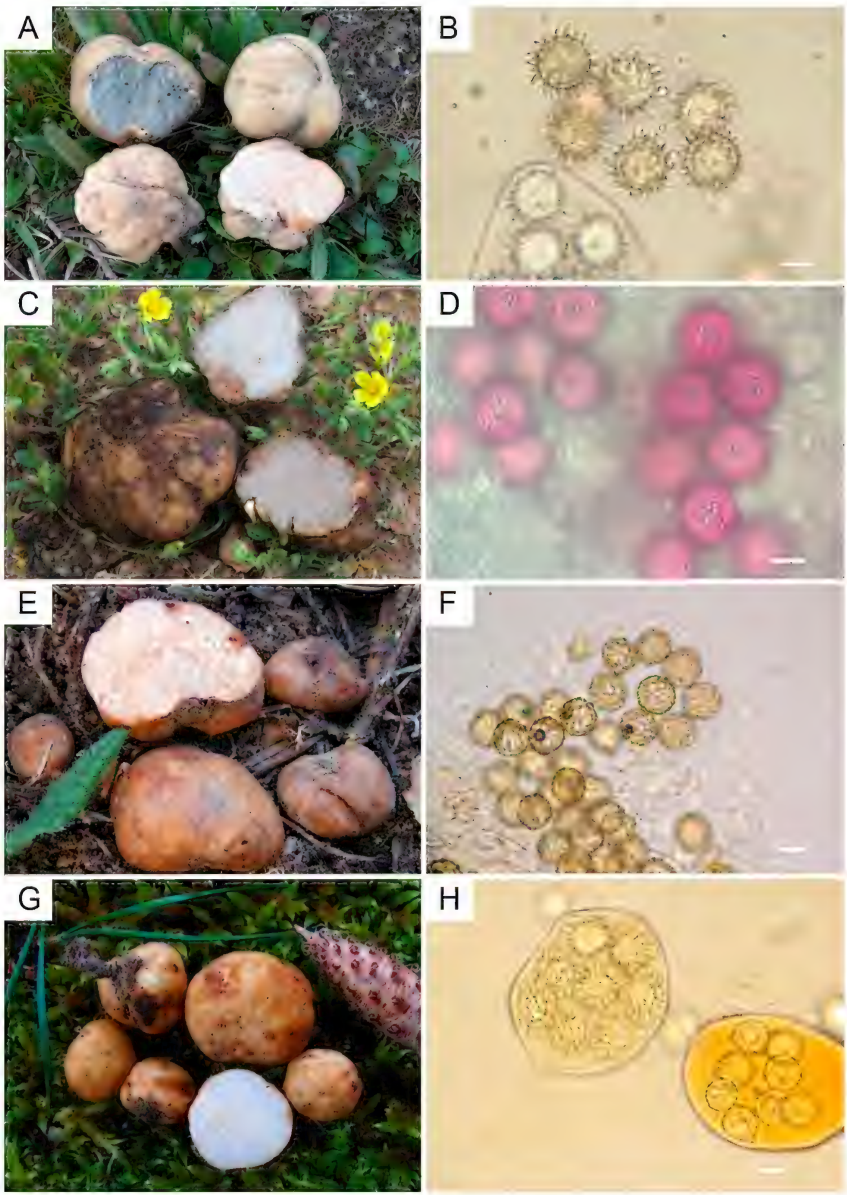


Plate 2. ***Terfezia extremadurensis*** (holotype, MUB Fung-0026): A, ascocarps; B, ascospores. ***T. eliocrocae*** (MUB Fung-0016): C, ascocarps; D, ascospores. ***T. alsheikhii*** (MUB Fung-0009): E, ascocarps; F, ascospores. ***T. pini*** (holotype, MUB Fung-0027): G, ascocarps; H, ascospores. Bars = 10 µm.

T. goffartii (25 µm without ornamentation) differentiates that species from *T. pini*. Despite the ascocarps of *T. pini* and *T. leptoderma* resemble each other, their spore ornamentations differ: the Tulasne brothers described the spicules of *T. leptoderma* as isolated (Tulasnes & Tulasne 1951), not crested nor forming a sub-reticulum on the spore surface. Moreover, the exclusive preference for burned oak or pine habitats has not been described for *T. leptoderma*.

Terfezia pseudoleptoderma Bordallo, Ant. Rodr. & Muñoz-Mohedano, **sp. nov.**
MycoBank MB561595 Plate 3a,b

Differs from *T. leptoderma* by its spore spicules with symmetric bases.

Type: Spain, Burgos, Villafranca Montes de Oca, Puerto de la Pedraja, 12 May 2008, leg F. Sáinz (**Holotype**, MUB Fung-0028).

Etymology: referring to the morphological similarity with *Terfezia leptoderma.*

Ascomata globose, round, quite regular, ≤2 cm diam., smooth when immature, partially rough when mature; initially cream colour, maturing to reddish-brown. Peridium smooth or slightly rough, inseparable from the gleba; cream colour, darkening where exposed to air. Gleba initially whitish, with the fertile tissue forming translucent greyish-blue islets surrounded by white veins or sterile tissue. Asci 8-spored; globose to ovoid. Ascospores spherical, 19–23 µm diam. (including ornamentation), spines separate, blunt, 2–5 µm long with bases asymmetric, ≤1 µm diam.; initially hyaline, yellowing in age.

Habitat — associated with cistaceous plants near pine and oak forests.

Additional collection examined: **SPAIN**: **Extremadura**, Cáceres: Valdecañas, 2010, Muñoz-Mohedano (MUB Fung-0039).

Comments — The ascocarps of *T. pseudoleptoderma* (Clade 5) are similar in size to *T. leptoderma,* which differs in the symmetric bases of the spore spicules.

In general, we noticed considerable confusion among authors who identify specimens as *T. leptoderma* that are much bigger than those described by Tulasne brothers.

Terfezia fanfani Mattir., Malpighia 14: 71. 1900. Plate 3c,d

Ascomata globose, round, regular, occasionally lobed, usually with mycelial remnants, 2–5 cm diam., smooth or slightly rough; firm; usually odourless. Peridium smooth, slightly rough, inseparable from the gleba, thin, 200–700 µm thick; initially white, soon becoming reddish brown, darkening in maturity with black maculae present in sun-exposed zones exposed; white in cross-section; pseudo-parenchymatous structure formed by rounded prismatic cells. Gleba initially white, then fertile tissue in islets becoming pale pink, then olive green, finally blackish grey when very mature. The gleba is always surrounded by white sterile tissue. Asci primarily 8-spored, sessile; subglobose, elongated

and ovoid, 70–80 × 55–70 µm diam. with 1 µm thick walls. Ascospores spherical with spines, 19–23(–25) µm diam. (including ornaments), initially hyaline, smooth, with a large central drop; in age ochre yellow and decorated with sharp thin elongated conic spines (2–)3–4(–5) µm long with 1 µm diam. bases (the spines not joined through the bases).

Habitat — acidic grasslands associated with *Tuberaria guttata* from the end of March to the end of April.

collections examined: **SPAIN**: **Extremadura**, Cáceres: Belvis, 2009, Muñoz-Mohedano (MUB Fung-0032); Garrovilla, 2004, A. Mateos (MUB Fung-0008); Valdecañas, 2001, Muñoz-Mohedano (MUB Fung-0004); Badajoz: Quintana de Serena, 2009, M. Romero (MUB Fung-0033); **Castile & León**, Valladolid: Cortas de Blas, 1998, Aurelio García (MUB Fung-0011).

Comments — *Terfezia fanfani* is recognized by its reddish colour, its growth with *Tuberaria guttata,* and its spores decorated with long and isolated spines. Initially hypogeous, it later it rises to the surface. Sharing both habitat and fruiting season with *T. arenaria*, *T. fanfani* fruits before *T. arenaria,* usually preferring saltier and less deep soils.

In size, *T. fanfani* seems better to match the Clade 6 specimens that we examined as well as those cited by other authors (Calonge et al. 1977; Ceruti 1960; Janex-Favre et al. 1988; Moreno et al. 1986). Thus for both phylogenetic and morphological reasons, we believe this species is independent.

Terfezia albida Ant. Rodr., Muñoz-Mohedano & Bordallo, **sp. nov.** Plate 3e,f

MycoBank MB561596

Differs from all other spiny-spored *Terfezia* spp. in its larger ascomata, its white peridium, and its spermatic odour.

Type: Spain, Albacete, El Ballestero, 2 May 2009, leg A. Rodríguez (**Holotype**, MUB Fung-0029).

Etymology: referring to the external whitish colour.

Ascomata hypogeous to partially emergent at maturity, 2–4 cm across, 3–4 cm high, subglobose to turbinate, pulvinate, often with tapered, sterile base, white at first, becoming light cream, often black spots on the sun-exposed parts or where handled, greenish with age on injured areas, smooth. Peridium 200–500 µm thick, poorly delimited, white in cross section, pseudoparenchymatous, composed of subglobose cells of variable size, hyaline and thin-walled in the innermost layers, yellowish and with thicker walls in the outermost layers. Gleba solid, fleshy, succulent, white at first, maturing to grayish green pockets of fertile tissue separated by whitish, sometimes with pink spots, sterile veins. Spermatic odour, stronger in young specimens. Asci inamyloid, subglobose to ovate, sessile or short-stipitate, 70–85(–90) × 55–70 µm, walls 1 µm thick, with 6–8 irregularly disposed spores, randomly arranged in the gleba. Ascospores

globose, (18–)19–22(–23) µm diam (median = 20 µm) including ornament, 14–17(–18) µm (median = 16 µm) without ornament, hyaline, smooth and uniguttulate at first, by maturity yellow ochre and ornamented with conical, blunt, straight spines, sometimes cylindrical and curved, sometimes truncated, separate, 2–3 µm long, 1–2 µm wide at the base, sometimes connected to form a pseudo-reticulum.

Ecology & distribution — southeastern Iberian Peninsula, limited to arid and semiarid areas in calcareous alkaline soils, associated with *Helianthemum* spp., from late April to mid May.

Additional collections examined: **SPAIN**: **Castile-La Mancha**, Albacete: Albacete, 2009, A. Rodríguez (MUB Fung-0037).

Comments — *Terfezia albida* (Clade 7) differs from other spiny-spored *Terfezia* species in its larger average size, white peridial colour, and spermatic odour. It is the only spiny-spored *Terfezia* species associated with *Helianthemum* spp. in alkaline soils. *Terfezia eliocrocae* (described above) and *T. claveryi* share the same habitat but differ in their reticulate spores.

Clade 7 includes taxa with different morphologies, distributions, and host plants from its sister clade, Clade 8 (where *T. olbiensis* is found). The two clades are separated by a 72% bootstrap value (Plate 1). Only after wide sampling we were able to place *T. albida* in Clade 7 (associated with cistaceous plants) and *T. olbiensis* in Clade 8 (associated with pine and oak hosts).

Terfezia olbiensis Tul. & C. Tul., G. Bot. Ital.1(2(7–8)): 60. 1845. Plate 3G,H

Ascomata globose, round, regular, rarely presents pseudostipe, 2–5 cm across, smooth. Peridium smooth, inseparable from the gleba, thin, 300–500 µm thick, not clearly delimited; initially cream, becoming brown, frequently with black maculae where exposed to the sun or bruised, white in cross-section; pseudoparenchymatous structure formed by ± rounded thin-walled hyaline cells of different sizes that yellow and become prismatic towards the periphery. Gleba initially white, then fertile tissue forming small grey (later greenish grey) islets surrounded by salmon-tinged white sterile tissue. Asci sessile to occasional on a short thick peduncle, 8-spored, ellipsoidal to ovoid, citrus-shaped, 60–90 × 50–60 µm with 1–2 µm thick walls; dextrinoid when immature. Ascospores spherical and spiny, 15–19 µm (including ornament) diam., initially hyaline, smooth, and with a great central drop, when mature ochre yellow and covered by pointy thin conical 1–2 (–2.5) µm long (base = 1 µm) spines (some truncated) that are sparse and not joined through the base.

Habitat — *Terfezia olbiensis* grows in limestone and clayey pine and oak woodlands without *Helianthemum* spp. from mid-March to mid-April. It is the first *Terfezia* species to produce ascocarps in limestone soils.

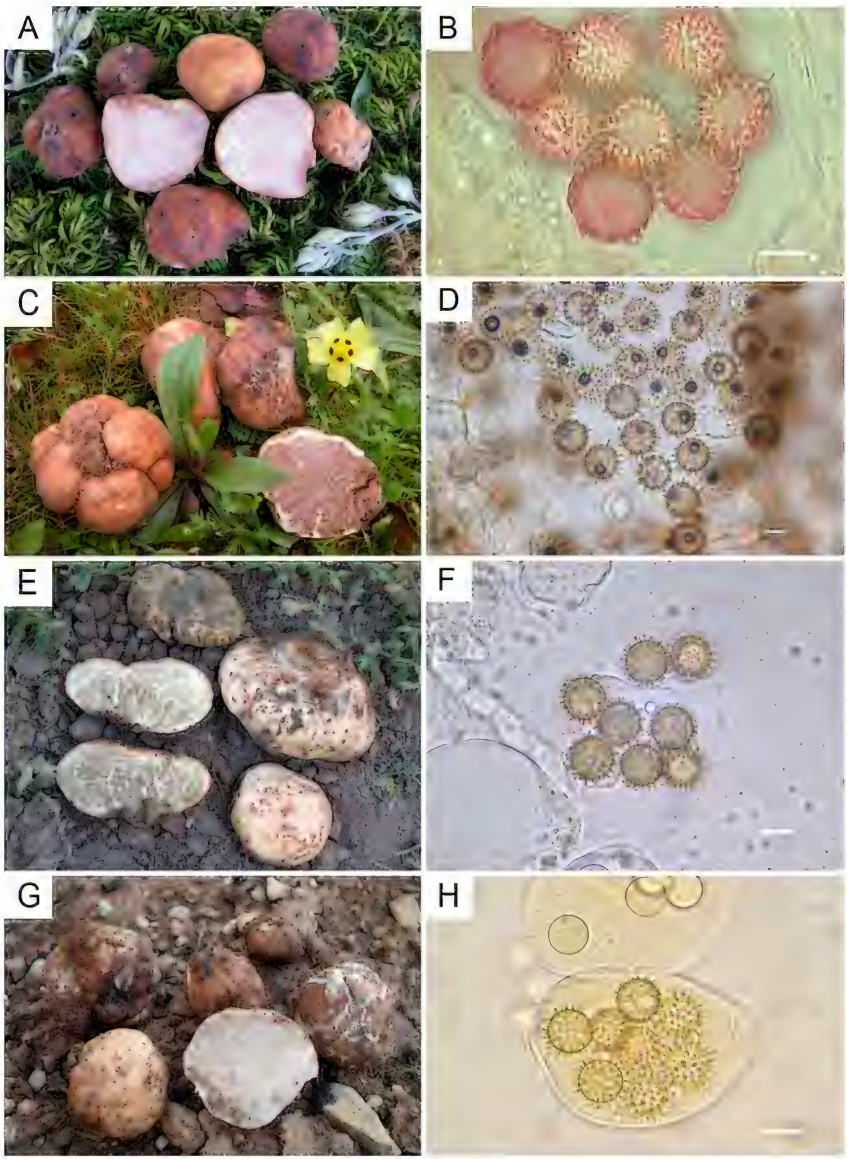


Plate 3. ***Terfezia pseudoleptoderma*** (holotype, MUB Fung-0039): A, ascocarps; B, ascospores. ***T. fanfani*** (MUB Fung-0032): C, ascocarps; D, ascospores. ***T. albida*** (holotype, MUB Fung-0029): E, ascocarps; F, ascospores. ***T. olbiensis*** (MUB Fung-0031): G, ascocarps H, ascospores. Bars = 10 μm.

COLLECTIONS EXAMINED: **SPAIN: CASTILE–LA MANCHA**, ALBACETE: Masegoso, 2009, A. Rodríguez (MUB Fung-0031); **CASTILE & LEÓN**, VALLADOLID: Santa Espina, 1998, Aurelio García (MUB Fung-0013); **VALENCIA**: Onteniente, 2009, F. García (MUB Fung-0030).

COMMENTS — Initially hypogeous, *T. olbiensis* later rises to the surface, where it remains firm. The fruitbodies usually are parasitized underground by larvae or eaten by rabbits, probably because they appear early in the year, when there is greater humidity and less sunlight. The odor is distinctive (somewhat rotten); the flavour is less than other edible *Terfezia* species. The most distinguished characteristic is the reduced length of its spines, always shorter than 2.5 µm.

Although similar to *T. leptoderma, T. olbiensis* has a bigger ascocarp (hazelnut- to walnut-sized) and smaller ascospores. Clade 8 taxa have different morphological and distribution characteristics from the other clades, which is also supported by the phylogenetic analyses with a 72% bootstrap value (PLATE 1). Therefore, we conclude that Clade 8 is formed by *T. olbiensis*.

Discussion

All the species described herein produce similar sized spiny to reticulate ascospores, which has led to the belief that they represent only one species. Our exhaustive comparison of the ascocarps and ascospores and comprehensive ITS-rDNA sequence analyses support recognition of several species.

Our results agree with those of Díez et al. (2002), who recognized three clades separated by a high statistical support (89%, 98%) based on morphological (gleba structure, size of ascospore spicules, ascospore size) and biological (acidic vs. alkaline soils, cistaceous vs. pine–oak hosts) characters. We propose Clade 4 for *Terfezia pini* based on data from Barriuso et al. (2008; see GenBank DQ386140). Moreover, a new sequence deposited by Rincón (unpublished data: GenBank FJ013064) belongs to Clade 5. In their later phylogenetic analyses of *Terfezia* spp., Ferdman et al. (2005) clearly separate DNA sequences AF276679, AF276678, AF387657, and AF387648 into two strongly supported different clades (100/99 bootstrap value) as *T. leptoderma* and *T. olbiensis* (Clade 8). This scenario is contrary to Malençon's opinion (see Díez et al. 2002, Moreno et al. 1986) that *T. olbiensis* represents an immature form of *T. leptoderma*.

The group covering species diagnosed by a spiny episporium (Clades 1, 4, 5, 6, 7 and 8) is paraphyletic, suggesting that episporium ornamentation is not a homologous character. Therefore, we do not consider episporium ornamentation a good taxonomic character, in contrast to other authors such as Mattirolo (1900).

Moreover, although AF396864 was classified as *T. leptoderma* (Díez et al. 2002), our analyses place it in a different clade by a relatively high bootstrap value (73/77). More sequences are needed to confirm its identity.

Our phylogenetic analyses corroborate the conclusion by Díez et al. (2002) that fungus-host specialization and soil tolerance are the keys that allow us to separate different species (clades). The monophyly of the proposed clades (species) (Plate 1) is congruent with the morphological (Plates 1 and 2) and biological (Table 1) data reported for the studied taxa.

Distributed throughout temperate and subtropical regions of the northern hemisphere, *Cistaceae* show the highest genus and species diversity in the Mediterranean floristic region (Guzmán & Vargas 2009). The Iberian Peninsula hosts a high diversity of *Cistaceae* and high number of mycorrhizal fungal species. Recent studies suggest that *Helianthemum almeriense* strongly depends on the presence of a fungal symbiont in its roots for survival (Morte et al. 2010). The Iberian Peninsula seems to be a desert truffle reservoir and further study and sampling are necessary in these ecosystems.

As recently highlighted by Bidartondo et al. (2008), we also emphasize the importance of an accurate identification of specimens whose DNA sequences have been subsequently registered in public databases, such as GenBank. Mistakes made in species identification can lead to erroneous and false conclusions. Checking the databases with contrasted data is necessary to prevent the perpetuation of errors when identifying new specimens.

Key to examined *Terfezia* species

Ascospore measurements include ornamentation.

1a. Fertile tissue does not create islets or reticulate ascospores 2
1b. Fertile tissue creates islets and spiny ascospores 3
2a. Ascospores with spines ..... ***T. extremadurensis*** (Clade 1)
2b. Ascospores reticulate 4
3a. Ascospores <20 µm and spines <2.5 µm ..... ***T. olbiensis*** (Clade 8)
3b. Ascospores ≥20 µm 5
4a. White gleba ..... ***T. eliocrocae*** (Clade 2)
4b. Pink gleba ..... ***T. alsheikhii*** (Clade 3)
5a. Spines <3 µm ..... ***T. albida*** (Clade 7)
5b. Spines >3 µm 6
6a. Crested spines ..... ***T. pini*** (Clade 4)
6b. Isolated spines 7
7a. Spines blunt with asymmetric base; ascomata <2 cm diam ..... ***T. pseudoleptoderma*** (Clade 5)
7b. Spines pointed with symmetric base; ascomata 2–5 cm diam .. ***T. fanfani*** (Clade 6)

Acknowledgments

This work has been supported by project CGL2011-29816 (MINECO-FEDER, Spain). The authors are grateful to Prof. Gabriel Moreno (Univ. Alcalá de Henares, Madrid) and

Prof. Giuseppe Venturella (Università di Palermo, Italy) for their presubmission reviews and Dr. Shaun Pennycook and Dr. Lorelei L Norvell for their huge effort and help in the text edition. They also thank Mr. A. Bordallo for his help with the English revision of the manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.209

Volume 124, pp. 209–217 *April–June 2013*

Hypochnicium pini, a new corticioid basidiomycete in East Asia

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Abstract — A new species, *Hypochnicium pini*, is described based on morphological and molecular characters. This species was collected in Seoul, Korea, and Hubei, China. It belongs to the *H. punctulatum* group and has a morphology quite similar to its sister taxon, *H. cremicolor*. However, the basidiome thickness, cystidial size, and ITS region sequences differentiate *H. pini* from *H. cremicolor*. Detailed description and illustration of *H. pini* are presented and a key to Korean species of *Hypochnicium* is provided.

Key words — phylogeny, *Polyporales*, taxonomy

Introduction

Hypochnicium J. Erikss. is a basidiomycete genus of wood-rotting fungi. It is characterized by resupinate effused adnate basidiomes, a margin that is usually not especially differentiated, monomitic hyphal system, hyaline thin- to thick-walled hyphae, cystidia present or absent, more or less suburniform 4-spored basidia normally with a basal clamp, and ellipsoid to subglobose smooth or ornamented basidiospores with thickened cyanophilous walls (Eriksson & Ryvarden 1976). More than 30 species have been described worldwide, and a core group around the type, *H. bombycinum* (Sommerf.) J. Erikss., has been shown to be monophyletic when *Hyphoderma* species are used as outgroup (Paulus et al. 2007; Tellería et al. 2010).

Traditionally, northern European *Hypochnicium* species were subdivided into six morphological groups (Eriksson & Ryvarden 1976), and within each group, species delimitation was troublesome because of rather similar morphological features. Recently, molecular phylogenetic analysis has been used to delimit species in *Hypochnicium*. Nilsson & Hallenberg (2003) first used ITS region sequences to differentiate species within the *H. punctulatum* group [*H. albostramineum* (Bres.) Hallenb., *H. cremicolor* (Bres.) H. Nilsson & Hallenb., *H. punctulatum* (Cooke) J. Erikss., *H. wakefieldiae* (Bres.) J. Erikss.] and clearly distinguished the species which complemented morphology and crossing test results. Several additional species belonging to this complex have been described: *H. cystidiatum* Boidin & Gilles from Africa (Boidin & Lanquetin 1971), *H. aotearoae* B.C. Paulus et al. from New Zealand (Paulus et al. 2007), *H. guineense* Tellería et al. from Equatorial Guinea (Tellería et al. 2010), and *Hypochnicium patagonicum* Gorjón & Hallenb. from Chilean Patagonia (Gorjón & Hallenberg 2012).

Six *Hypochnicium* species have been reported from Korea: *H. bombycinum*, *H. detriticum* (Bourdot) J. Erikss. & Ryvarden, *H. eichleri* (Bres. ex Sacc. & P. Syd.) J. Erikss. & Ryvarden, *H. lundellii* (Bourdot) J. Erikss., *H. punctulatum*, and *H. vellereum* (Ellis & Cragin) Parmasto (Jung 1991, 1994, 1996; Lim et al. 2010). *Hypochnicium eichleri* and *H. punctulatum* were reported by Jung (1991, 1996), who identified the species referring to *Corticiaceae* of North Europe (Eriksson & Ryvarden 1976). However, Nilsson & Hallenberg (2003) proved that "*H. punctulatum*" as described in Eriksson & Ryvarden (1976) was actually *H. cremicolor* (The type of *H. punctulatum* has bigger basidiospores than *H. cremicolor*). Moreover, *H. eichleri* has been synonymised with authentic *H. punctulatum*. Therefore the Korean report of "*H. punctulatum*" should be corrected to *H. cremicolor*; and the Korean record of *H. eichleri* should be updated to *H. punctulatum*.

Recently, the national biological inventory project (governed by the National Institute of Biological Resources) is underway in Korea. During the investigation of indigenous fungi, we found a *Hypochnicium* specimen sharing features with *H. cremicolor* but with smaller cystidia. Our further investigation revealed two specimens from China with the same deviating characteristics. Based on morphological and molecular data, we describe these three specimens as a new species.

Materials & methods

Collection and morphological examination

The corticioid basidiome was collected from Gungsan (Mt.), Seoul, Korea and air-dried. The dried specimen was used for macro- and microscopic examination. The specimen was mounted in 1% cotton blue, 5% KOH, 1% phloxine, and Melzer's

reagent (IKI) (Largent et al. 1977) and observed at up to 1000× using Olympus BX51 light microscope. Munsell color (2009) was followed as the color standard. More than 30 basidiospores were examined. The specimen was deposited at National Institute of Biological Resources, Korea (KB). The Chinese specimens were borrowed from National Museum of Natural Science, Taiwan (TNM) and examined as above.

Phylogenetic analysis

For the Korean specimen, genomic DNA extraction, ITS region PCR, and sequencing were carried out according to Jang et al. (2012). For the Chinese specimens, the revised method of Cenis (1992) was used for genomic DNA extraction. PCR and sequencing were performed according to Jang et al. (2012). The sequences obtained in this study were deposited under the GenBank accession no. JX217823, KC282470–282472. The sequences were proofread and compared with the reference sequences in GenBank. Closely related *Hypochnicium* sequences were retrieved from GenBank. Only one sequence was selected when two or more sequences had the same nucleotides; *H. geogenium* (Bres.) J. Erikss. (GenBank AF429426) was included as outgroup based on the phylogenetic tree in Tellería et al. (2010). The sequences were aligned using MAFFT 6.885 (Katoh & Toh 2008) with L-INS-i method. The aligned dataset was viewed and manually edited using MacClade 4.08 (Maddison & Maddison 2005). Phylogenetic tree was constructed using MrBayes 3.2 (Ronquist et al. 2012). Two independent runs with 8 chains were operated with 1,000,000 generations and every 100th tree was saved. In this process, the best-fit model was searched by MrModeltest 2.3 (Nylander 2004) under AIC criterion and GTR + I model was applied. The graphical presentation of log likelihood value was checked and the first 25% of the trees were discarded. The 50% majority rule consensus tree was produced with the rest 75% of the trees. The potential scale reduction factors (PSRF) were reasonably close to 1.0 for all parameters. Posterior probabilities were calculated to support nodes. The tree was viewed using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Taxonomy

Hypochnicium pini Y. Jang & J.J. Kim, **sp. nov.** FIGS 1–3

MycoBank MB 800807

Differs from *Hypochnicium cremicolor* by its thinner basidiome and its smaller cystidia.

TYPE: Korea. Seoul, Gangseo-gu, Gungsan (Mt.), 37°34′25″N 127°50′25″E, on bark of *Pinus densiflora* Siebold & Zucc., 23 Sep 2008, Yeongseon Jang KUC20081023-04 (**Holotype**, KB NIBRFG0000107453; GenBank JX217823).

ETYMOLOGY: Growing on pines (*Pinus*).

Basidiome resupinate, effused, membranaceous, pale yellow (2.5Y8/2, 2.5Y8.5/2) when dry, up to 0.1 mm thick; hymenophore smooth to porulose (hypochnoid); margin not differentiated. Hyphal system monomitic; hyphae with clamps, ramified, hyaline, 3.8–5.8 μm wide; subhymenial hyphae thin-walled while the basal ones are thick-walled, walls up to 1.5 μm thick, loosely interwoven. Cystidia subcylindrical, slightly tapering but with an obtuse apex, enclosed

Fig. 1. *Hypochnicium pini* (NIBRFG0000107453, holotype). Basidiocarp. Scale bar = 1 cm.

within the hymenium or projecting up to 40 µm above the basidial layer, thin-walled, variable in size, 40–80 × 5.5–8.5 µm. Basidia clavate, sinuous, 28–39 × 5.5–7.5 µm, with four sterigmata and a basal clamp. Basidiospores broadly ellipsoid to subglobose, 5.5–6.9 × 4.6–5.9 µm, mean 6.1 × 5.2 µm, verrucose in 1% cotton blue and 1% phloxine, smooth in 5% KOH, thick-walled, some with a few guttula, spore wall cyanophilous, IKI–.

Ecology & distribution — China and Korea, on *Pinus* spp.

Additional specimens examined:

***Hypochnicium pini*: CHINA, Hubei**, Wuhan, Mt. Lion, alt. 30 m, 30°29′N 114°21′E, on trunk of *Pinus* sp., 12 November 2008, S.H. Wu 0811-75 (TNM F0023763; GenBank KC282471); Wu 0811-77 (TNM F0023765; GenBank KC282472).

***Hypochnicium cremicolor*: CHINA, Yunnan**, Tali, Tsangshan, alt. 2800 m, on trunk of *Pinus armandii* Franch., 19 October 2000, S.H. Wu & S.C. Wu 0010-180 (TNM F0012210; GenBank KC282470).

Remarks — *Hypochnicium pini* is very similar to *H. cremicolor*. However, *H. cremicolor* has a thicker basidiome (0.1–0.3 µm) and larger cystidia (70–150 × 7–10 µm; Eriksson & Ryvarden 1976, as "*H. punctulatum*"). Phylogenetic analysis also revealed the close relationship between the two species, but there was sufficient genetic differentiation to separate them (Fig. 3).

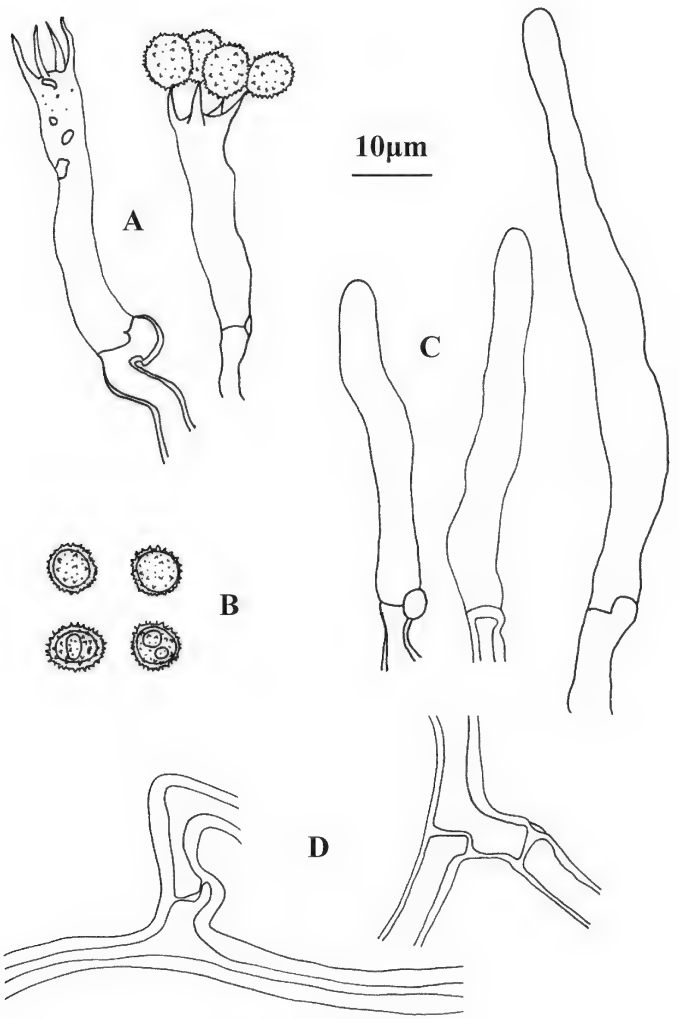

Fig. 2. *Hypochnicium pini* (NIBRFG0000107453, holotype).
A, basidia; B, basidiospores; C, cystidia; D, thick-walled basal hyphae.

Phylogeny

The amplified ITS region sequences of *Hypochnicium pini* NIBRFG0000107453 from Korea, and F0023763 and F0023765 from China were 578 bp. There were two nucleotide differences between the Korean specimen and the Chinese specimens of *H. pini* and the sequences of Chinese specimens were identical.

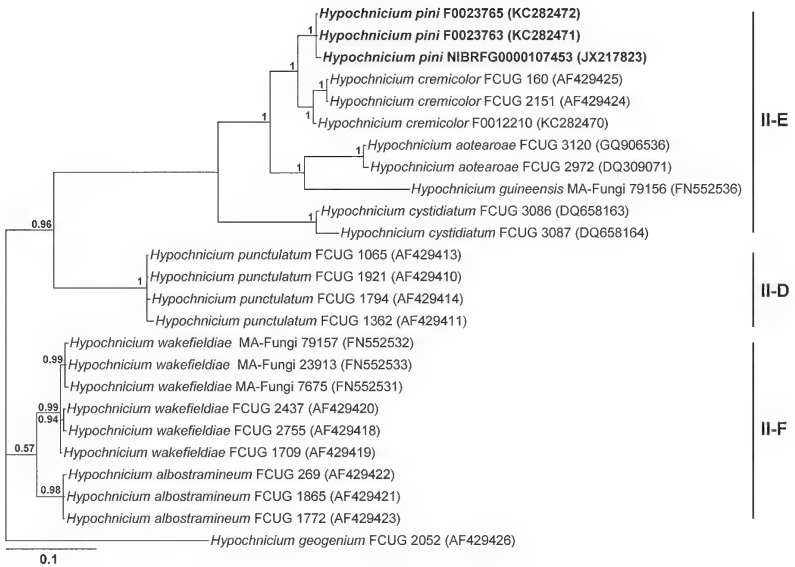
Hypochnicium pini F0023765 (KC282472)
Hypochnicium pini F0023763 (KC282471)
Hypochnicium pini NIBRFG0000107453 (JX217823)
Hypochnicium cremicolor FCUG 160 (AF429425)
Hypochnicium cremicolor FCUG 2151 (AF429424)
Hypochnicium cremicolor F0012210 (KC282470)
Hypochnicium aotearoae FCUG 3120 (GQ906536)
Hypochnicium aotearoae FCUG 2972 (DQ309071)
Hypochnicium guineensis MA-Fungi 79156 (FN552536)
Hypochnicium cystidiatum FCUG 3086 (DQ658163)
Hypochnicium cystidiatum FCUG 3087 (DQ658164)
Hypochnicium punctulatum FCUG 1065 (AF429413)
Hypochnicium punctulatum FCUG 1921 (AF429410)
Hypochnicium punctulatum FCUG 1794 (AF429414)
Hypochnicium punctulatum FCUG 1362 (AF429411)
Hypochnicium wakefieldiae MA-Fungi 79157 (FN552532)
Hypochnicium wakefieldiae MA-Fungi 23913 (FN552533)
Hypochnicium wakefieldiae MA-Fungi 7675 (FN552531)
Hypochnicium wakefieldiae FCUG 2437 (AF429420)
Hypochnicium wakefieldiae FCUG 2755 (AF429418)
Hypochnicium wakefieldiae FCUG 1709 (AF429419)
Hypochnicium albostramineum FCUG 269 (AF429422)
Hypochnicium albostramineum FCUG 1865 (AF429421)
Hypochnicium albostramineum FCUG 1772 (AF429423)
Hypochnicium geogenium FCUG 2052 (AF429426)
II-E
II-D
II-F
0.1

FIG. 3. 50% majority rule consensus tree of the *Hypochnicium punctulatum* group inferred by ITS region sequences using bayesian analysis. The tree was rooted by *H. geogenium* FCUG 2052. Numbers above branches indicate posterior probabilities. *H. pini* is indicated by bold type. The clades presented in the tree follow those resolved by Telleria et al. (2010).

By the nucleotide BLAST, the closest match was *H. cremicolor* FCUG160 from Denmark AF429425 (93%) and *H. cremicolor* FCUG2151 from Spain AF429424 (93%) (555 out of 596 positions are identical for Korean specimens and 557 out of 596 positions for Chinese specimens). In order to fully understand the relationship within the *H. punctulatum* group, *H. pini* sequences were aligned with 22 closely related *Hypochnicium* sequences obtained from GenBank including *H. cremicolor* Wu 0010-180 from China, and with *H. geogenium* FCUG2052 as outgroup. The aligned ITS dataset of 25 taxa comprised 586 characters including gaps. The 50% majority rule consensus tree using bayesian analysis is shown in FIG. 3.

Each species was well resolved in the tree as indicated by high posterior probability values. Three main groups were recovered which correspond to the subclades II-D, II-E, and II-F in Tellería et al. (2010). They all have ornamented basidiospores, but there are just minute morphological characteristics which distinguish each subclade. *Hypochnicium pini* was included in subclade II-E with four formerly described species – *H. aotearoae*, *H. cremicolor*, *H. guineense*, and *H. cystidiatum*. *Hypochnicium pini* NIBRFG0000107453 from

Korea was monophyletic with *H. pini* F0023763 and F0023765 from China with high posterior probability (1.0 p.p.); and *H. cremicolor* Wu 0010-180 from China was monophyletic with formerly sequenced *H. cremicolor* FCUG160 and FCUG 2151, also with high support (1.0 p.p.), and it was 98% similar to them in BLAST search (588 out of 596 positions are identical).

Discussion

Morphologically, species within II-E clade have similar characteristics. *Hypochnicium pini* is characterized by its pale yellow basidiocarp, hypochnoid hymenophore, thick-walled basal hyphae, one type of cystidium and broadly ellipsoid to subglobose spores, which are very similar to *H. cremicolor* (Figs 1, 2). They both have the basal thick-walled hyphae, one type of cystidium, and ornamented basidiospores which are similar in size [*H. pini*: 5.5–6.9 × 4.6–5.9 µm; and *H. cremicolor*: (5–)5.5–6.5 (–7.5) × 4.5–5 µm (Eriksson & Ryvarden 1976) or 6–6.5 × 5–5.5 µm (Nilsson & Hallenberg 2003)]. The hosts of *H. pini* are *Pinus* spp. while *H. cremicolor* has been reported from both broadleaved and coniferous trees including *Pinus* spp. (Eriksson & Ryvarden 1976, Bernicchia & Gorjón 2010). The obvious morphological differences are the thickness of basidiome and the size of cystidia (Table 1). *Hypochnicium aotearoae* and *H. cystidiatum* differ from *H. pini* by having two types of cystidia, and *H. guineense* differs by larger basidiospores (Tellería et al. 2010).

Table 1. Morphological characteristics of Korean species of *Hypochnicium* s.l.

Species	Cystidia (µm)	Basidia (µm)	Basidiospores (µm)
H. bombycinum (Jung 1994)	None	40–50 × 6–8	Smooth, 8–12 × 6–8
H. cremicolor (Jung 1996, as "*H. punctulatum*")	60–130 × 9–12	25–30 × 6.5–7.5	Ornamented, (5–)6–6.5 × 4.5–5.5
H. detriticum (Jung 1994)	60–100 × 6–10	15–20 × 5	Smooth, 4.5–6 × 3.5–4
H. lundellii (Jung 1994)	None	25–30 × 5–6	Smooth, 5.5–6 × 4.5–5
H. pini (this paper)	40–80 × 5.5–8.5	28–39 × 5.5–7.5	Ornamented, 5.6–6.9 × 4.8–5.9
H. punctulatum (Jung 1991, as "*H. eichleri*")	90–110 × 6–10	25–35 × 7.5–9	Ornamented, 8–9(–10) × 6–7.5
H. vellereum (Lim et al. 2010)	None	42–65 × 4–7.42	Ornamented, 6.8–7.4 × 5.7–6.2

In conclusion, we propose that the unknown *Hypochnicium* species collected from Gungsan (Mt.) in Korea and from Hubei in China represents a new species, *Hypochnicium pini*, minutely distinguished by morphological data, but with distinct genetic differences towards other species in this complex. We also update the list of Korean species of *Hypochnicium* sensu lato (Table 1).

Key to the Korean species of *Hypochnicium* s.l.

1. Without cystidia 2
1. With cystidia 4
2. Spores smooth 3
2. Spores globose, with irregularly uneven or finely rough surface ..... *H. vellereum*
3. Spores broadly ellipsoid to ovoid, 8–12 × 6–8 µm ..... *H. bombycinum*
3. Spores ellipsoid, 5.5–6 × 4.5–5 µm ..... *H. lundellii*
4. Spores smooth ..... *H. detriticum*
4. Spores ornamented 5
5. Spores longer than 8 µm ..... *H. punctulatum*
5. Spores shorter than 8 µm 6
6. Basidiome 0.1–0.3 mm thick, cystidia 60–130 × 9–12 µm ..... *H. cremicolor*
6. Basidiome <0.1 mm thick, cystidia 40–80 × 5.5–8.5 µm ..... *H. pini*

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10011390) and was funded by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea. We are grateful to Dr. Sheng-Hua Wu (National Museum of Natural Science, Taiwan) for providing Chinese specimens. We are grateful to Dr. Sergio Pérez Gorjón and Dr. Leif Ryvarden for their valuable suggestions on the manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.219

Volume 124, pp. 219–229 *April–June 2013*

Taxonomic studies on *Mucor inaequisporus*, isolated for the first time in South America

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Abstract— *Mucor inaequisporus* (*Mucorales*, *Mucoromycotina*) was isolated from *Syzygium cumini*, a common introduced tree species in Brazil. It is distinguished from similar species by producing straight or undulate sporangiophores with randomly distributed irregular swellings. The columellae are mostly pyriform at 25 °C, but oblong, conic, ellipsoid, and obovoid columellae were also found. The sporangiospores vary in size and shape, with a minor proportion exhibiting an irregular shape. ITS-based phylogenetic analyses reveal that *M. inaequisporus* is closely related to *M. amphibiorum* and *M. nederlandicus*. This species can grow between 20 and 30 °C with optimal development at 25 °C, but variations in the shape of sporangiospores and columellae caused by incubation under different temperatures were observed. No growth was detected at 10 and 40 °C.

Key words— DNA sequencing, growth rate, zygomycete

Introduction

Mucor Fresen., which belongs to the subphylum *Mucoromycotina* (Hibbett et al. 2007), is characterized by the formation of non-apophysate sporangia (with wet or dry walls when mature) on top of simple or branched sporangiophores that emerge from the substrate (Benny 2006). Taxa of this genus have been isolated from soil, dung, decaying fruits, and plants (White et al. 2006, Santiago & Souza-Motta 2008, Santiago et al. 2011), while others are considered the causal agent of cutaneous zygomycosis in humans (Álvarez et al. 2011). Several species are able to produce enzymes with biotechnological applications (Alves et al. 2002a), and some are used to prepare fermented food (Hesseltine 1983, Abe et al. 2004).

Previous studies have treated *Mucor* as polyphyletic and highlighted the difficulty of establishing subgeneric groups (O'Donnell et al. 2001, Voigt & Wöstemeyer 2001, Jacobs & Botha 2008). Although over 300 species are cited in the literature (Jacobs & Botha 2008), the actual number of species may range only from 50 to 75 (Gherbawy et al. 2010). After revising several *Mucor* species, Schipper (1978) reduced the proposed taxa to 39 species, four varieties, and 11 forms. Subsequently, another 17 species have been described as new taxa (Mehrotra & Mehrotra 1979; Mirza et al. 1979, Subrahamanyam 1983, Chen & Zheng 1986, Schipper 1989, Schipper & Samson 1994, Watanabe 1994, Pei 2000, Alves et al. 2002b, Jacobs & Botha 2008, Álvarez et al. 2011, Madden et al. 2012).

Mucor inaequisporus was first described by Dade (1937) from *Spondias mombin* L. fruits in Aburi, Ghana. Since then, a few records of this species from several fruits have been reported. Morphologically, *M. inaequisporus* is mainly characterized by the highly variable form and size of columellae and sporangiospores (Schipper 1978).

The present study describes *Mucor inaequisporus* isolated from a fruit of *Syzygium cumini* (L.) Skeels (= *Eugenia jambolana* Lam.; *Myrtaceae*), commonly found in the State of São Paulo and known locally as 'Jambolão' or 'Jamelão.' We discuss the influence of temperature, light, and culture media on growth and on morphology of *M. inaequisporus.*

Materials and methods

Isolation and morphological identification of *M. inaequisporus*

Mature fruits of *S. cumini* were collected on the campus of the Federal University of São Carlos (21°59′02″S 47°52′55″W), São Paulo, Brazil. In February 2009 (URM 6532) and February 2010 (URM 6533), mycelium fragments were removed directly from growing fruits under a stereomicroscope (Leica EZ4) and transferred to Petri dishes with potato dextrose agar (PDA) (Benny 2008) supplemented with chloramphenicol (Neo Fenicol – Neo Química) (80 mg L^{-1}). Plates were left on a bench at room temperature (28 ± 2 °C) for 7 days in alternating light and dark periods. The strains were subcultured in order to obtain pure cultures. Identification was based on macroscopic (color, aspect and diameter of the colonies) and microscopic characters according to Dade (1937) and Schipper (1978).

Influence of temperature, culture media, and light on the growth and morphology of *M. inaequisporus*

Pure cultures were grown in malt extract agar (MEA) and PDA (Benny 2008) at 15, 20, 25, 30, 35, and 40 °C for 7 days under light and dark periods. Mycelial fragments were removed from cultures to prepare wet mounts using KOH (3%) and studied under bright-field microscopy (BFM) (Carl Zeiss Axioscope 40). Color designation followed Watling (1969). The morphological characteristics were also studied using Environmental Scanning Electron Microscopy (ESEM). Agar blocks retrieved from

Table 1. *Cokeromyces* and *Mucor* species included in the molecular analysis

Species	Isolate	GenBank No.
Cokeromyces recurvatus Poitras	CBS 158.50^{T}	DQ118986
M. amphibiorum Schipper	CBS 763.74^{T}	FJ455861
M. bainieri B.S. Mehrotra & Baijal	CBS 293.63	JF299222
M. circinelloides Tiegh. (as *M. circinelloides* f. *circinelloides*)	CBS 195.68^{NT}	DQ118991
M. ellipsoideus Ed. Álvarez et al.	UTHSC 02-2090^{T}	FN650647
M. flavus Bainier	CBS 230.35	EU484282
M. fragilis Bainier	CBS 236.35	FN650655
M. fuscus Bainier	CBS 132.22^{T}	FN650653
M. genevensis Lendn.	CBS 114.08^{T}	EU484275
M. guilliermondii Nadson & Filippov	CBS 174.27^{T}	JF299231
M. hiemalis f. *corticola* (Hagem) Schipper	CBS 106.09	AY243950
M. hiemalis Wehmer f. *hiemalis*	CBS 201.65	DQ118992
M. inaequisporus	CBS 255.36^{T}	JN206177
	CBS 351.50	JN206178
	URM 6532	JQ014007
M. indicus Lendn.	CBS 226.29^{T}	DQ118994
M. irregularis Stchigel et al.	CBS 103.93^{T}	DQ119006
M. lusitanicus Bruderl. (as *M. circinelloides* f. *lusitanicus*)	CBS 108.17^{T}	FN650644
M. mucedo Fresen.	CBS 109.16	EU484199
M. nederlandicus Váňová	CBS 735.70	JF299219
M. nidicola Madden et al. (as *M. hiemalis*)	CBS 638.67	JF299220
M. piriformis A. Fisch.	CBS 169.25	EU484276
M. plumbeus Bonord.	CBS 111.07	AF412290
M. racemosus Fresen.	CBS 260.68^{T}	DQ118996
M. ramosissimus Samouts.	CBS 135.65^{NT}	FN650643
M. variosporus Schipper	CBS 837.70^{T}	HM623322
M. velutinosus Ed. Álvarez et al.	UTHSC 06-1667	FN650652
M. zonatus Milko	CBS 148.69^{T}	JF299232

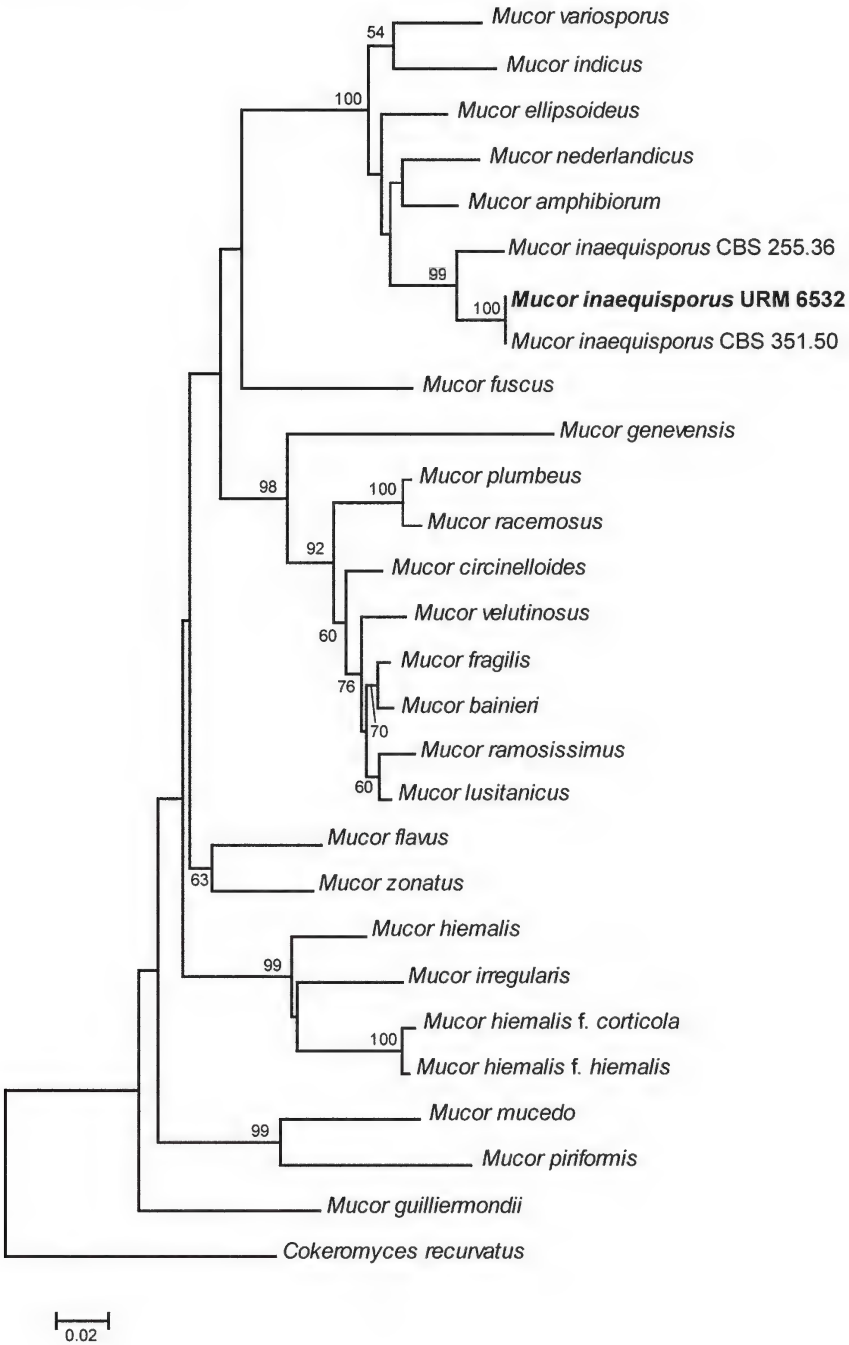
CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; UTHSC = Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA; URM = University Recife Mycology, Recife, Brazil

^{NT} = neotype strain; ^{T} = type strain

cultures were mounted in microscope slides for direct observation with a scanning electron microscope (FEI Quanta™ 250).

Phylogenetic analyses

DNA was extracted following Gerardo et al. (2004). Cultures of *M. inaequisporus* URM 6532 were cultured in malt broth (2%) at 25 °C for seven days and the mycelium was freeze-dried and disrupted using liquid nitrogen. Genomic DNA was recovered using CTAB and purified with 70% ethanol. PCR primer pair ITS4 and ITS5 was used to amplify the ITS1-5.8S rDNA-ITS2 region (White et al. 1990). Both forward and reverse sequences were generated in ABI3500 (Applied Biosystems). Sequences were assembled in Bioedit v. 7.0.5.3 (Hall 1999) and the consensus was aligned with sequences of several *Mucor* species retrieved from GenBank using Muscle v. 3.6 (Edgar 2004) (Table 1). A neighbor-joining tree was inferred in MEGA5 (Tamura et al. 2011) under the


Mucor variosporus
Mucor indicus
Mucor ellipsoideus
Mucor nederlandicus
Mucor amphibiorum
Mucor inaequisporus CBS 255.36
Mucor inaequisporus URM 6532
Mucor inaequisporus CBS 351.50
Mucor fuscus
Mucor genevensis
Mucor plumbeus
Mucor racemosus
Mucor circinelloides
Mucor velutinosus
Mucor fragilis
Mucor bainieri
Mucor ramosissimus
Mucor lusitanicus
Mucor flavus
Mucor zonatus
Mucor hiemalis
Mucor irregularis
Mucor hiemalis f. corticola
Mucor hiemalis f. hiemalis
Mucor mucedo
Mucor piriformis
Mucor guilliermondii
Cokeromyces recurvatus
54
100
99
100
98
100
92
60
76
70
60
63
99
100
99
0.02

maximum-composite likelihood algorithm to calculate genetic distances. Robustness was calculated using 1000 bootstrap pseudo-replicates, and the tree was rooted with *Cokeromyces recurvatus* (Table 1).

Results

Phylogenetic Analysis

The size of the ITS region of *M. inaequisporus* URM 6532 is 536 bp. BLASTn comparisons with sequences deposited at the GenBank revealed that the ITS sequence of this strain is 92% identical with a sequence labeled as *Mucoraceae* LM042 (EF060714), 91% identical with another undescribed *Mucor* species (AB638465), 90% identical to the sequence of *M. amphibiorum* (FJ455861), 95% identical with *M. inaequisporus* (CBS 255.36), and 100% identical with *M. inaequisporus* (CBS 351.50). The phylogenetic relationship among *Mucor* species based on the ITS region is illustrated in Fig. 1. *Mucor guilliermondii* appears as the basal species, phylogenetically distant from other species in the genus. *Mucor inaequisporus* was genetically distant from *M. mucedo*, the type species of the genus, and clustered in a well-supported clade (100% bootstrap value) with *M. nederlandicus*, *M. amphibiorum*, *M. ellipsoideus*, *M. variosporus*, and *M. indicus*. Another well-supported clade (98% bootstrap value) included *M. ramosissimus*, *M. lusitanicus*, *M. bainieri*, *M. fragilis*, *M. circinelloides*, *M. velutinosus*, *M. plumbeus*, *M. racemosus*, and *M. genevensis*. Phylogenetic analysis indicates only distant relatedness within two pairs of morphologically similar species, *M. genevensis*/*M. hiemalis* and *M. nederlandicus*/*M. guilliermondii*.

Taxonomy

Mucor inaequisporus Dade, Trans. Br. Mycol. Soc. 21(1): 25. 1937. Pl. 1

Colonies grow rapidly on MEA, filling the entire Petri dish (9 cm diam. and 10 mm height) after 72 hours at 25 °C in the dark. The colony ranges from an obverse intense yellow (8G to luteus 51) with yellow to olive-gray spots, corresponding to sporangia, and reverse yellow (8G), particularly at the point of inoculum. Rhizoids were short to long (≤600 µm in length), poorly branched, with or without septa. A sweet aroma was produced. Sporangiophores 9–30 µm in diam., with or without yellow droplets, simple or with long and short sympodial branches (3–10 times, mostly after 6 days of incubation), erect, undulant, curved, sometimes with one or several randomly spaced septa and sometimes constricted next to the sporangia; phototropic,

Fig. 1. Neighbor-joining tree of *Mucor* spp. inferred from the ITS-rDNA region. The maximum-composite likelihood algorithm was used to calculate genetic distances. Numbers on branches are bootstrap support values obtained from 1000 pseudo-replicates (values under 50% are not shown). A sequence of *Cokeromyces recurvatus* was used to root the tree. *Mucor inaequisporus* examined in this work is denoted in bold. Scale bar indicates the number of base substitutions per site.

with yellow to chestnut contents and with incrusted walls. Some sporophores exhibit randomly distributed irregular swellings (≤47 µm in diam.), frequently gradually tapering towards the base. Sterile branches are common. Sporangia globose and subglobose to lightly depressed, yellow and turn brown with age, with finely echinulated, transparent and deliquescent walls that are more persistent in the smaller sporangia, (47.5–)70–130(–180) × (45–)60–120 (–165) µm; Columellae highly variable in shape, frequently pyriform (mostly in the larger ones), conic or oblong, but also ellipsoid, obovoid, subglobose, colorless or slightly yellow to chestnut; smooth walled with prominent collars, (45–)60–100(–140) × (35–)50–80(–120) µm; Sporangiospores hyaline with granular contents, highly variable in shape and size: majority elliptical (sometimes flattened at one side), 5–14(–22.5) × 3.5–10(–15) µm, also globose, subglobose (≤15 µm diam.), some irregular (≤24 × 15 µm). Chlamydospores and zygosporangia not observed.

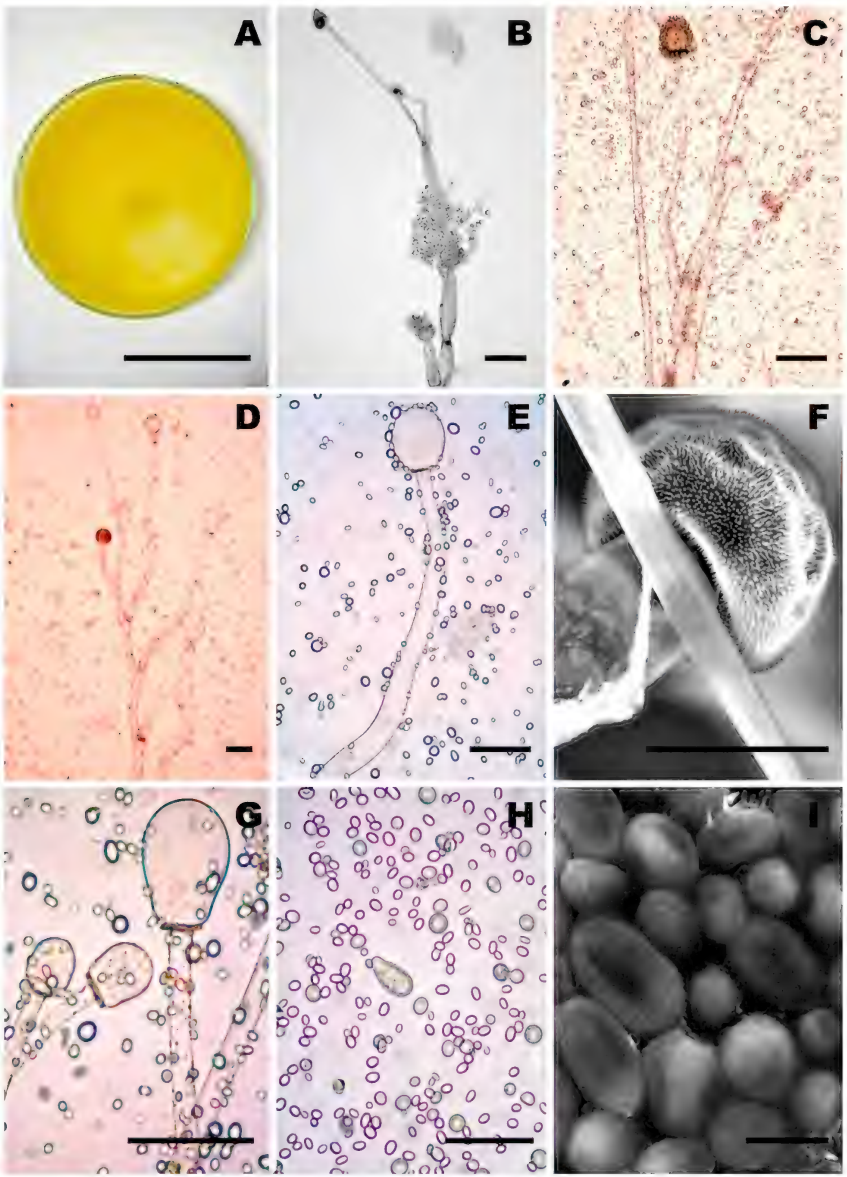
Influence of temperature, culture media and light on the growth and morphology of *M. inaequisporus*

Macroscopic and microscopic features on PDA were similar to those on MEA. The optimum growth temperature was 25 °C (colonies filled the entire Petri plate after 72 hours) with good growth and sporulation between 15 and 30 °C. Limited growth was recorded at 35 °C and no growth was observed at 10 and 40 °C. Colonies incubated at 30 °C grew well (9 cm diam. after 72 hours), but were shorter in height (6 mm) and the sporangiophores were more branched than at 25 °C. The columellae were mainly oblong and conical, rarely pyriform, and the sporangiospores were irregular in shape, predominantly globose and subglobose. Colonies incubated at 20 °C reached 9 cm in diam. after 72 hours. Such colonies were irregularly zonate, at raised in the center and then touching the entire lid of the Petri dish after 7 days. Abundant twisted sterile and coiled hyphae were observed in the aerial mycelium. Colonies were also irregularly zonate at 15 °C, reaching 9 cm diam. and 2 mm in height after 96 hours. Abundant twisted sterile hyphae were also observed in the aerial mycelium. Influence of light was not detected.

Specimens examined — **BRAZIL, São Paulo**: Campus of the Federal University of São Carlos, (21°59′02″S 47°52′55″W), on mature fruits of *Syzygium cumini*, 23.01.2009, leg. E.M. Canedo (URM 6532); 12.02.2010, leg. E.M. Canedo (URM 6533).

Habitat — Growing on fruits of *Artocarpus glaucus*, *Bouea macrophylla*, *Diospyros kaki*, *Flacourtia inermis*, *Musa paradisiaca*, *M. sapientum*, *Spondias mombin*, *Syzygium cumini*, and *Theobroma cacao* (Dade 1937, Boedijn 1959, CBS Database, IMI Database) and in the rhizosphere of root-knot nematode host plants (Zangeneh et al. 2007).

Distribution — Ghana, Indonesia, Iran, Malaysia, and Brazil. This is the first report for South America.



Pl. 1. *Mucor inaequisporus* grown in MEA at 25 °C for 7 days. A. Colony growing in a Petri dish. B–D. Branched sporangiophores, some with randomly distributed irregular swellings with sporangia and columellae. E. Single undulate sporangiophore with columella. F. Young sporangiophore. G. Columellae. H–I. Sporangiospores. Scale bars. A = 5 cm; B, D–F, H = 50 µm; C, G =100 µm; I = 5 µm. (A–E, G, H = pictures under BFM; F, I = pictures under ESEM.)

Discussion

Several studies have reported the variability of nucleotide sequences of the ITS-rDNA region as a reliable indicator to differentiate taxa of *Mucorales* at the species level, including taxa of *Mucor* (Iwen et al. 2002, Schwarz et al. 2006, Hoffmann et al. 2009). Strain URM 6532 shared an identical ITS sequence with strain CBS 351.50. According to our phylogenetic analysis, *M. inaequisporus* is genetically distinct from other described *Mucor* species. This species exhibited low percentages of similarity with *M. nederlandicus* (91%), with *M. amphibiorum* and *M. variosporus* (90%), and with *M. ellipsoideus* (89%). According to Walther et al. (2013), the ITS is a barcode marker for mucoralean species identification, despite the wide intraspecific variations found for some taxa. *Mucor inaequisporus* appears to have some ITS variability because strain URM 6532 showed a 95% similarity with strain CBS 255.36. Thus, our phylogeny is in agreement with the phylogeny presented in Walther et al. (2013).

Morphologically, *M. inaequisporus* is distinguished from other *Mucor* species by the production of sporophores that are straight, curved, or undulant, and with randomly distributed irregular swellings. The columellae are variable in form, being predominantly pyriform at 25 °C. Sporangiospores are extremely variable in size and shape at 25 °C. However, some features of our isolate, such as curved or undulant sporophores with irregular swellings, were not described by Dade (1937) and Schipper (1978). Columellae sizes reported in the literature (≤100 × 90 µm, Dade 1937; 83–75 µm, Schipper 1978) were smaller than our maximum measurements, whereas sporangiospores (≤25 × 20 µm, Dade 1937; ≤30 × 23 µm, Schipper 1978) were larger than our maximum measurements. According to Schipper (1978), different strains of *M. inaequisporus* may exhibit variable morphologic and physiologic features.

Our results show that *M. inaequisporus* exhibited growth from 15 to 30 °C. However, colonies incubated at 30 °C showed an increased number of branches. The columellae were mostly oblong and conical, rarely pyriform, and the sporangiospores were mostly globose, subglobose, and irregular in shape, different from the observed at 25 °C. Schipper (1975, 1976) has noted the influence of temperature on the shape of columellae and/or sporangiospores in other *Mucor* species such as *M. plasmaticus* Tiegh., *M. psychrophilus* Milko, and *M. racemosus* f. *chibinensis* (Neophyt.).

We report for the first time the occurrence of *M. inaequisporus* in Brazil, thereby expanding the knowledge of *Mucorales* distribution. Strains URM 6532 and URM 6533 were isolated from a common introduced tree species in Brazil, *S. cumini,* which belongs to the *Myrtaceae*. As this is the first report of a *Mucor* species in fruits in this country, this record adds to the twenty-four species of *Mucor* that have been isolated from soil and/or dung in Brazil (Santiago 2012).

Acknowledgments

This work was financially supported by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE). The authors would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for a scholarship to Enzo M. Canedo. We are also grateful to Rafael José Vilela, Diogo Xavier Lima and Dr. Luciana Bueno dos Reis Fernandes for technical assistance. We are in debt to Dra. Maria Inês Salgueiro Lima for the identification of plant specimens and to Dr. José Ivanildo de Souza and Dr. Matias Cafaro for manuscript review.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.231

Volume 124, pp. 231–238 *April–June 2013*

Molecular phylogeny reveals *Megacollybia virosa* is a *Cantharocybe*

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Abstract — Nuclear LSU rDNA sequence analysis unequivocally places an agaric currently designated as *Megacollybia virosa* in *Cantharocybe*. Based on this molecular evidence, *Cantharocybe virosa* comb. nov. is proposed. This transfer results in the recognition of a third distinct species of *Cantharocybe*. The collections from India form the first record of the genus outside the North American continent. Phenetically, *C. virosa* shares with the type species of the genus, *C. gruberi*, the clitocyboid habit, distinct cheilocystidia, convex pileus with an inrolled margin, subdecurrent to decurrent lamellae, thickset stipe, ellipsoid, thin-walled, hyaline, smooth and inamyloid basidiospores, clamped hyphae, subregular to regular lamellar trama, a cutis-type pileipellis disrupted to form trichodermal patches, pileipellis hyphae with plasmatic pigment, and cystidioid terminal cells. A key to the known species is provided.

Key words — *Agaricales*, *Basidiomycota*, systematics, taxonomy

Introduction

Cantharocybe is a little studied agaric genus (*Agaricales, Basidiomycota*) so far known only from North and Central America. The genus was described by Bigelow & Smith (1973) to accommodate a clitocyboid agaric with robust, yellowish basidiomata, lecythiform or mucronate cheilocystidia, oblong or subcylindric, smooth, inamyloid basidiospores, and clamped hyphae. The genus was originally typified by *Clitocybe gruberi* A.H. Sm. (Smith 1944) from Idaho based on a single sporocarp but subsequently collected from New Mexico, California, and Washington as well. According to Bigelow & Smith (1973), the combination of yellow pigmentation, the unusual cheilocystidia, and subcylindric basidiospores of this species warranted its transfer from *Clitocybe* to a new genus. *Cantharocybe* remained a monotypic genus for nearly three decades, but very recently, another species, *C. brunneovelutina* Lodge et al., was described from Belize (Ovrebo et al. 2011). Ovrebo et al. (2011)

emended the genus concept by widening the range of states of characters such as the texture and color of pileus surface and shape of both basidiospores and cheilocystidia. Although the distinctiveness of *Cantharocybe* as a genus is supported by molecular analyses within the *Agaricales*, the family within which *Cantharocybe* should be placed is uncertain (Binder et al. 2010, Ovrebo et al. 2011). *Cantharocybe* is currently placed among the basal genera of the hygrophoroid clade (i.e., *Hygrophoraceae*) based on molecular analyses with low support (Binder et al. 2010; Ovrebo et al. 2011).

Repeated collections of an agaric from various parts of Kerala State, India have been made in the last two decades. As these particular collections defied identification even to the genus level, it remained unpublished for quite a long time. Eventually it was described as *Megacollybia virosa* based solely on morphology (Manimohan et al. 2010). Lingering doubts about its identity prompted us to undertake a molecular analysis, which revealed that it is closely allied to the two species of *Cantharocybe* mentioned above. The results of our molecular analysis are presented and discussed here.

Materials & methods

DNA was extracted from authentic material (TENN063483) of *Megacollybia virosa* deposited at the Herbarium of the University of Tennessee. Detailed voucher information is available in Manimohan et al. (2010). DNA was extracted from dried tissue using the procedure described in Mata et al. (2007). The nuclear large subunit (LSU) ribosomal RNA region was amplified and sequenced with primers LROR and LR5. Sequences were edited in Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, Michigan), the segments with ambiguous bases trimmed from the ends, and similar sequences were searched in GenBank with the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) algorithm. Initial BLAST queries showed close affinities with *Cantharocybe brunneovelutina* (92% sequence identity) and *C. gruberi* (91% sequence identity). An LSU sequence dataset was assembled with sequences of taxa that showed >90% similarities with the newly generated sequence, additional sequences of representative members of genera (including outgroup *Phyllotopsis nidulans* (Pers.) Singer) selected based on Ovrebo et al. (2011), and sequences of representative species of *Megacollybia* (Hughes et al. 2007) were downloaded from GenBank and assembled into a dataset. The newly generated sequence was deposited in GenBank with accession number JX101471.

The LSU sequence dataset was manually aligned in CLUSTALX 2.1 and then examined in Se-Al 2.0a11 (Andrew Rambaut, University of Edinburgh, U.K. http://tree.bio.ed.ac.uk/software/seal). The region analyzed contained a total of 1452 bp. The alignment was deposited in TreeBase with submission number 12767 (http://purl.org/phylo/treebase/phylows/study/TB2:S12767). Maximum Parsimony (MP) analysis was conducted in PAUP* 4.0b10 (Swofford 2002) using a heuristic search option with TBR branch swapping, 1000 replicates using random step-wise addition, and holding one tree at each step. All minimal length trees were saved, and maxtrees was set as unrestricted. Bootstrap analysis (Felsenstein 1985) was carried out to evaluate support for

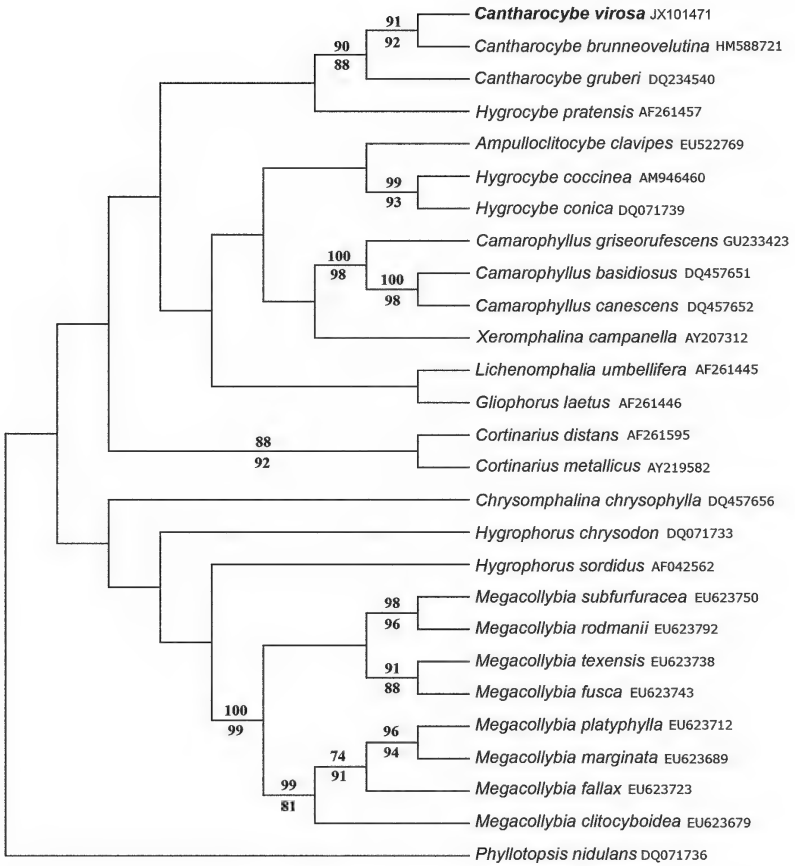

FIGURE 1. One of four equally parsimonious trees generated during the phylogenetic analyses of LSU rDNA sequences. Numbers above branches indicate MP bootstrap values and numbers below branches indicate BI clade credibility (posterior probability) values. Only values greater than 70% are shown. GenBank accession numbers are provided.

the branching topologies with a full heuristic search option on 1000 replicates. Bayesian inference (BI) analysis was done using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The general time reversible model with gamma distributed rate variation option was selected. Multiple independent analyses were done for 1 million generations with trees saved every 100 generations. Options for incremental heating scheme, chain number, and priors were set to MrBayes default settings. The first 1000 samples were burned before calculating posterior probabilities.

Manimohan et al. (2010) is followed for the morphological description. Color codes refer to Kornerup & Wanscher (1978).

Results

Megacollybia virosa is resolved in a clade with *C. brunneovelutina* and *C. gruberi* with strong branch support in both Bayesian and parsimony analyses (Fig. 1). The analysis also places *Hygrocybe pratensis* (Fr.) Murrill (≡ *Camarophyllus pratensis* (Fr.) P. Kumm.) as the sister group to *Cantharocybe* but with no support. Three species of *Camarophyllus* (= *Cuphophyllus*) were included in the analysis group with species of *Xeromphalina*, *Ampulloclitocybe,* and *Hygrocybe*. Although a relationship of these taxa with *Cantharocybe* is indicated, the placement is not well supported. Eight representative species of the genus *Megacollybia* form a distinct clade with strong support in all the maximum parsimony and maximum likelihood analyses. *Cantharocybe virosa* shows no close phylogenetic affinities with members of *Megacollybia*.

Discussion

DNA sequencing and analysis of the nLSU gene region supports a close phylogenetic relationship between *Megacollybia virosa* and the two known species of *Cantharocybe*. Although initially described as a species of *Megacollybia*, the distinctiveness of *M. virosa* had been recognized based on its abundant cheilocystidia of a unique morphology and the distinctive terminal elements of the pileipellis (Manimohan et al. 2010). The cheilocystidia in *M. virosa* feature a clavate, cylindrico-clavate, or ventricose basal part and an upper part extending into an elongated neck with or without a rounded capitulum (Fig. 2b). While this unique cystidial morphology is not characteristic of *Megacollybia*, it is seen in the type species of *Cantharocybe, C. gruberi*. Phenetically, apart from the morphology of cheilocystidia, *M. virosa* shares the following characters with *Cantharocybe*: robust clitocyboid basidiomata (Fig. 2a), convex pileus with an inrolled margin, subdecurrent to decurrent lamellae, thickset stipe, ellipsoid, thin-walled, hyaline, smooth and inamyloid basidiospores (Fig. 2c), clamped hyphae, subregular to regular lamellar trama, a cutis-type pileipellis disrupted to form trichodermal patches, and pileipellis hyphae with plasmatic pigment and cystidioid terminal cells. At the same time, it differs from both *C. gruberi* and *C. brunneovelutina* in having a grayish brown pileus surface. Additionally, *C. gruberi* differs in having much larger, narrowly elliptic to oblong basidiospores, whereas *C. brunneovelutina* has a unique cystidial morphology with a resemblance to basidia. According to Matheny et al. (2006), *C. gruberi* belongs to pluteoid clade and in Moncalvo et al.'s (2002) analysis, the relationships of *C. gruberi* remained unresolved although it formed a distinct clade with *Camarophyllus pratensis*. The placement of *Camarophyllus* (= *Cuphophyllus*) and *Cantharocybe* in the hygrophoroid clade has been indicated by Binder et al. (2010) and Ovrebo et al. (2011). The collections of *M. virosa* from India form the first report of a *Cantharocybe* species outside the North American continent.

Based on the results of the present molecular analysis, the following new taxonomic combination is proposed:

Cantharocybe virosa (Manim. & K.B. Vrinda) T.K.A. Kumar, **comb. nov.** Fig. 2

MycoBank MB800480

≡ *Megacollybia virosa* Manim. & K.B. Vrinda, Mycotaxon 111: 364. 2010.

Basidiomata medium-sized, fleshy, clitocyboid. Pileus 45–100 mm broad, convex, becoming broadly convex; surface light brown (6D5), brown (6E4), grayish brown (7E3), or dark gray (7F8), slightly darker at the center, pruinose to somewhat granular to the naked eye, with fine appressed scales under a lens, dry; margin inrolled when very young, becoming incurved and finally becoming straight, initially entire, becoming fissile with age. Lamellae adnate to decurrent, moderately crowded, with lamellulae in four to eight tiers, up to 9 mm deep, whitish to yellowish white (1A2); edges smooth to finely fimbriate under a lens. Stipe 20–75 × 5–23 mm, central or at times slightly excentric, terete to slightly compressed, almost equal with a dilated apex, solid, with white basal mycelium; surface dull white with fine, light brown (6D5), grayish

Figure 2. *Cantharocybe virosa* (TENN063485, holotype).
a. Basidiomata; **b**. Cheilocystidium; **c**. Basidiospore. Scale bars: a = 1 cm; b, c = 10 µm.

brown (7E3), or dark gray granular squamules concentrated towards the base and disappearing easily when handled. Context ≤20 mm thick, white. Odour strong and unpleasant. Spore print white.

Basidiospores 6.5–11(–12) × 5–7 µm, subglobose to ellipsoid, thin-walled, smooth, with refractive guttules, inamyloid. Basidia 23–56 × 7.5–11 µm, cylindrico-clavate to clavate, thin-walled, hyaline, with granular contents, 4-spored; sterigmata ≤5 µm long. Cheilocystidia abundant, 20–63 × 5.5–9 µm, clavate, cylindrico-clavate, or ventricose, majority with a small capitellum on a slender neck up to 35 µm long, often septate, thin-walled, hyaline to pale yellowish, apex sometimes covered with glutinous exudates; edges of lamellae sterile. Pleurocystidia absent. Lamellar trama regular to subregular, hyphae 3–15 µm wide, hyaline to pale yellowish, thin-walled, inamyloid. Pileal trama interwoven; hyphae 2–20 µm wide, slightly inflated, hyaline to pale yellowish, thin-walled. Pileipellis mostly a cutis of highly interwoven agglutinated hyphae, occasionally disrupted with trichodermal patches, hyphae 3–10 µm wide, thin- to slightly thick-walled, often with a grayish brown plasmatic pigment; terminal elements cystidioid, 23–85 × 3–10 µm, similar to cheilocystidia in all aspects, often with a greyish brown plasmatic pigment. Stipitipellis a highly disrupted and irregular cutis with agglutinated trichodermal patches of ascending to erect hyphal elements, hyphae 2–21 µm wide, thin- to slightly thick-walled, with gray to dark grayish plasmatic and membrane pigment; terminal elements cystidioid, similar to cheilocystidia in all aspects. Clamps frequent on all hyphae.

Habitat: On soil or on mud walls, often associated with roots of coconut trees in a tropical climate, solitary or in caespitose clusters, May to August.

Specimens examined — **INDIA, Kerala State**, Calicut District, Puthiyangadi, 31 July 2005, T.K. Arun Kumar AK373 (TENN063483, GenBank, JX101471); 10 August 2005, T.K. Arun Kumar AK378 (TENN063476); 12 June. 2006, T.K. Arun Kumar AK395 (TENN063485, holotype); 21 June 2006, T.K. Arun Kumar AK397 (TENN063486); Thiruvananthapuram District, Plamood, 30 May 1998, C.K. Pradeep TBGT4310 (TENN063480); Vizhinjam, 11 December 2000, C.K. Pradeep TBGT5249 (TENN063479); 23 May 2002, C.K. Pradeep TBGT5526 (TENN063482); Muttada, 9 July 2006, K.B. Vrinda TBGT9804 (TENN063481); 28 August 2006, K.B. Vrinda TBGT9938 (TENN063484).

Key to the species of *Cantharocybe*

1a. Pileus dark brown; cheilocystidia resembling basidia with 1–4 sterigma-like apical appendages, extending at oblique angles and frequently swollen or capitate at the apex; basidiospores 9–9.5 × 5.5–6 µm, Belize, Central America (tropical) ..... ***C. brunneovelutina***

1b. Pileus yellow or pale grayish brown; cheilocystidia lecythiform or sometimes with a mucronate apex **2**

2a. Pileus yellow; basidiospores (13.5)14–15(16) × 3 5.5–6 µm, narrowly elliptic to oblong, western North America (temperate) ..... ***C. gruberi***

2b. Pileus pale grayish brown; basidiospores 6.5–11(–12) × 5–7 µm, subglobose to broadly ellipsoid, India (tropical)..... ***C. virosa***

Cantharocybe virosa is known to be toxic, capable of inducing severe gastro-intestinal upset when eaten (Manimohan et al. 2010). Nothing is known about the potential toxicity of the two American species.

Acknowledgments

We are greatly indebted to Prof. Karen W. Hughes and Prof. Ronald H. Petersen for their kind help in sequencing the DNA of *Cantharocybe virosa*. We thank Dr. M. Catherine Aime for valuable comments on the identity of the species and for a critical review of the manuscript. We are also grateful to Dr. P. Brandon Matheny for a critical review of the manuscript.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.239

Volume 124, pp. 239–245 *April–June 2013*

New record of *Scedosporium dehoogii* from India

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Abstract — Three strains of *Scedosporium* were isolated from Pimpri (Pune), Maharashtra, India, during a survey of soil fungi in industrial areas. Morphological and sequence (ITS1-5.8S-ITS2 rDNA) analyses confirm them to be *Scedosporium dehoogii* (reported here as a new record for India) and *S. apiospermum*.

Key words — disease, *Microascaceae*, phylogeny, scedosporiosis, taxonomy

Introduction

Scedosporium Sacc. ex Castell. & Chalm. (teleomorph *Pseudallescheria* Negroni & I. Fisch.; *Microascaceae*) are cosmopolitan fungi found in soil, sewage, and water, some of which can cause infection in humans. Five medically important *Scedosporium* species — *S. apiospermum* Sacc. ex Castell. & Chalm., *S. aurantiacum* Gilgado et al., *S. boydii* (Shear) Gilgado et al., *S. dehoogii*, and *S. prolificans* (Hennebert & B.G. Desai) E. Guého & de Hoog (Gilgado et al. 2005, 2008) — cause various diseases (broadly termed as scedosporiosis; Guarro et al. 2006, Cortez et al. 2008) that mostly infect immunocompromised patients, causing mycetoma, septic arthritis, osteomyelitis, meningitis, brain abscesses, etc. (Cooley et al. 2007). In India, there have been several reports of *Scedosporium* infection (Lingappa & Lingappa 1962, Acharya et al. 2006, Gopinath et al. 2010, Nath et al. 2010, Mathew et al. 2012). During the present study we isolated one *Scedosporium apiospermum* strain and two *S. dehoogii* strains. Our survey of the literature and fungal checklists (Bilgrami et al. 1991, Jamaluddin et al. 2004) indicates that *S. dehoogii* is a new record for India.

Materials & methods

The fungi were isolated from soil collected from industrial region of Pimpri (Pune), Maharashtra, India, on 23 December 2011. Isolated strains were cultured on five media

obtained from Hi-Media (India) — 2% malt extract agar (MEA), potato dextrose agar (PDA), Czapek Dox agar (CDA), potato carrot agar (PCA), oatmeal agar (OA) — at 10–40±0.2°C in the dark. Morphological characteristics were observed on PDA at 25±0.2°C (7 d). Cultures have been deposited at the Microbial Culture Collection (MCC), National Centre for Cell Science, Pune, India as *S. apiospermum* MCC 1042 and *S. dehoogii* MCC 1043 and MCC 1044, and a specimen of MCC 1044 has been deposited in the Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute, Pune, India. Microscopic characters were studied in lactophenol stained with cotton blue (Hi-Media, India) using Nikon YS100 microscope (Nikon, Japan). Microphotographs were taken on Olympus BX53 (Olympus Corporation, Japan) fitted with ProgRes C5 camera (Jenoptik, USA). Samples were coated with platinum on Jeol minor coater (Jeol-JFC 1600) for examination on a Jeol-JSM 6360A scanning electron microscope at 10 kV. Fungal DNA was isolated from pure 7 d old cultures with beads and lysis buffer in 2 ml Eppendorf tubes according to Sharma & Gräser (2011). Total DNA was checked on gel and NanoDrop spectrophotometer (NanoDrop, USA). PCR was conducted with a Gene Amplifier PCR System 9700 (Perkin Elmer, USA) using ITS1 and ITS4 primers for ITS1-5.8S-ITS2 region (White et al. 1990). A 25 µl mixture containing 10 U Taq polymerase buffer (New England Biolabs), 2 mM dNTPs, 10 pM primers, 1 unit Taq polymerase (New England Biolabs), and 10 ng DNA was amplified following a protocol of initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, final extension at 72°C for 10 min. The PCR product was checked on 1.0% agarose gel and purified by PEG-NaCl (Polyethylene glycol-NaCl). Sequencing was done on ABI 3730xl Automated Sequencer using 'ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit' (Perkin Elmer Applied Biosystems Division, Foster City, CA). An NCBI Blastn search for ITS region was conducted for sequence similarity (Altschul et al. 1990). Sequences of MCC 1042, MCC 1043 and MCC 1044 were generated in lab and other sequences retrieved from GenBank and deposited in European Molecular Biology Laboratory (EMBL) database. The sequences were aligned and edited in Clustal W of Molecular Evolutionary Genetics Analysis (MEGA 5.0) computer program (Higgins et al. 1994, Tamura et al. 2011). Phylogenetic analyses were done by Kimura 2-parameter model using Neighbour Joining (NJ) statistical method. Gaps and missing data was removed by complete deletion option of the software. The bootstrap consensus tree was inferred from 1000 replicates and bootstrap values ≤50% were removed from the tree (Saitou & Nei 1987; Tamura et al. 2011).

Taxonomy

Scedosporium dehoogii Gilgado, Cano, Gené & Guarro, J. Clin. Microbiol. 46(2): 768. 2008. PLATE 1

The PDA colonies, which reached 32 mm diam. after 7 d at 25±0.2°C, were radially zonate, dense, cottony to lanose, grayish-white to white and with a whitish lobate or irregular and fimbriate margin; the reverse was yellowish fading towards margin and with radial zones also visible. Other strain was effused, white to pale gray, fast growing, 75 mm after 7 d at 25±0.2°C on PDA

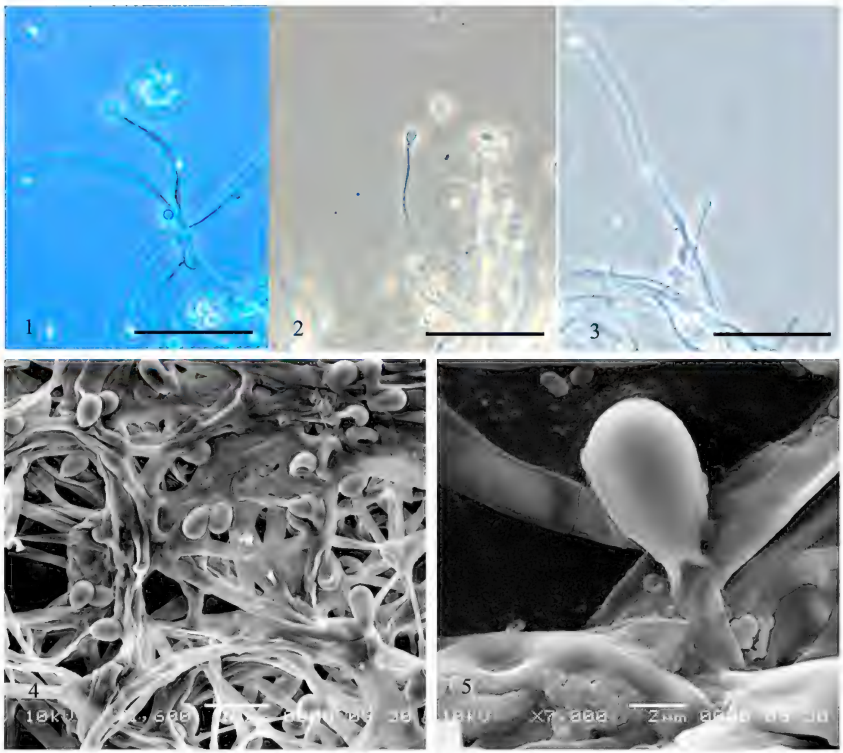

Plate. 1. *Scedosporium dehoogii* MCC 1044. Fig. 1–3. Conidiogenous cells with conidia. Fig. 4. SEM of mycelial mat with conidia. Fig. 5. SEM of sessile conidia. Scale bars: 1–3 = 50 µm; 4 = 10 µm; 5 = 2 µm.

with colorless reverse. Solitary conidiophores were subhyaline smooth-walled, 10–54 × 1–1.4 µm, and produced pale brown, obovoid or ellipsoidal, 5.9–6.2 × 3.6–4.4 µm conidia. Sessile conidia subhyaline, thick-walled, mostly obovate, (6.9–)7.3(–7.8) × (3.5–)3.8(–4.0) µm. Hyphae hyaline to subhyaline, 1.2–2.5 µm. A teleomorph was not observed even after 60 d of incubation.

Distribution & habitat: Pimpri (Pune), Maharashtra, India (soil from industrial area). (First described from garden soil in Barcelona, Spain.)

Material examined: **INDIA**, **Maharashtra State**, Pimpri (Pune), 18°37(07.04(N 73°48(13.43(E, soil, 23.XII.2011, Rohit Sharma, culture S9-H (AMH-9561; MCC 1044; GenBank HF570054); culture S9-D (MCC 1043; GenBank HF570053).

Results & discussion

Both *Scedosporium dehoogii* strains had similar optimum and maximum growth temperatures, but the MCC 1043 PDA colonies grew around two times faster than those of MCC 1044 (Plate 2). *Scedosporium apiospermum* MCC

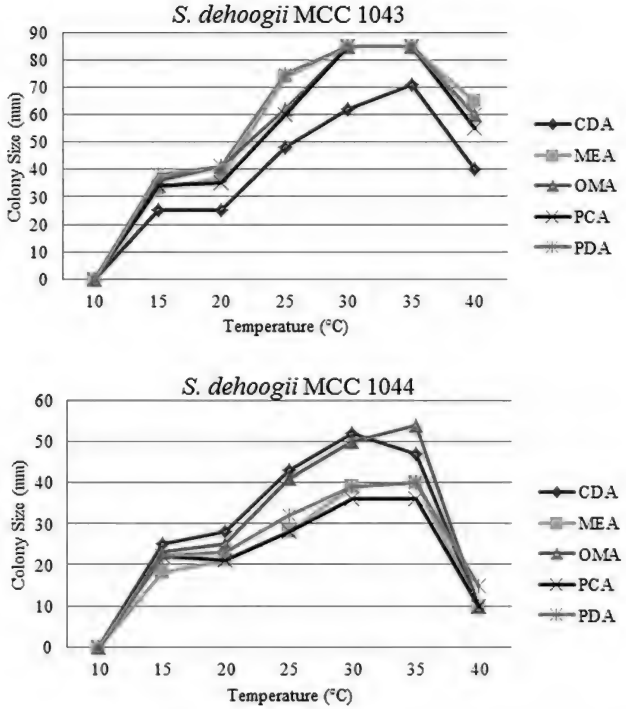

Plate. 2. Temperature-growth relationship of *Scedosporium dehoogii* MCC 1043 and MCC 1044 on Malt Extract Agar (MEA), Czapek Dox Agar (CDA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA) (mm diam. of 7-d old colonies).

1042 had an optimum temperature of 35±0.2°C on OMA (15±0.2°C minimum and 40±0.2°C maximum). The sessile conidia of *S. dehoogii* MCC 1044 are narrower than in the ex-type culture CBS 117406 (5–6 µm; Gilgado et al. 2008). ITS sequences of 62 strains retrieved from GenBank were aligned with the sequences from our *S. apiospermum* and *S. dehoogii* strains. Of the 545 bp used to construct the phylogeny, 434 were conserved, 107 variable, 76 parsimony-informative, and 31 singletons. The NJ-phylogenetic analysis clustered MCC 1043 and MCC 1044 in the *S. dehoogii* species group, which forms a separate branch clearly distinguishing it from other *Scedosporium* species (Plate 3).

Scedosporium apiospermum and *S. prolificans* have been reported several times from India and are associated with various types of infections in both immunocompromised and immunocompetent patients (Gopinath et al. 2010, Nguyen & Raychaudhuri 2011, Patil et al. 2011, Mathew et al. 2012, Kaur et al. 2013, Matlani et al. 2013); however, *S. dehoogii* is reported for the first time from

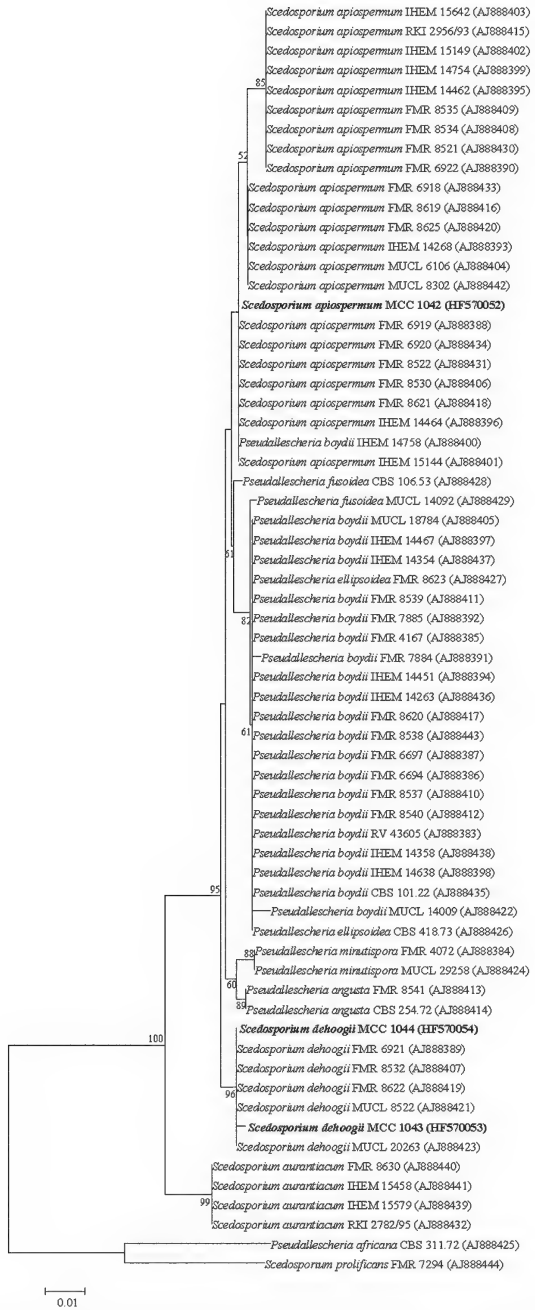
Scedosporium apiospermum IHEM 15642 (AJ888403)
Scedosporium apiospermum RKI 2956/93 (AJ888415)
Scedosporium apiospermum IHEM 15149 (AJ888402)
Scedosporium apiospermum IHEM 14754 (AJ888399)
Scedosporium apiospermum IHEM 14462 (AJ888395)
Scedosporium apiospermum FMR 8535 (AJ888409)
Scedosporium apiospermum FMR 8534 (AJ888408)
Scedosporium apiospermum FMR 8521 (AJ888430)
Scedosporium apiospermum FMR 6922 (AJ888390)
Scedosporium apiospermum FMR 6918 (AJ888433)
Scedosporium apiospermum FMR 8619 (AJ888416)
Scedosporium apiospermum FMR 8625 (AJ888420)
Scedosporium apiospermum IHEM 14268 (AJ888393)
Scedosporium apiospermum MUCL 6106 (AJ888404)
Scedosporium apiospermum MUCL 8302 (AJ888442)
Scedosporium apiospermum MCC 1042 (HF570052)
Scedosporium apiospermum FMR 6919 (AJ888388)
Scedosporium apiospermum FMR 6920 (AJ888434)
Scedosporium apiospermum FMR 8522 (AJ888431)
Scedosporium apiospermum FMR 8530 (AJ888406)
Scedosporium apiospermum FMR 8621 (AJ888418)
Scedosporium apiospermum IHEM 14464 (AJ888396)
Pseudallescheria boydii IHEM 14758 (AJ888400)
Scedosporium apiospermum IHEM 15144 (AJ888401)
Pseudallescheria fusoidea CBS 106.53 (AJ888428)
Pseudallescheria fusoidea MUCL 14092 (AJ888429)
Pseudallescheria boydii MUCL 18784 (AJ888405)
Pseudallescheria boydii IHEM 14467 (AJ888397)
Pseudallescheria boydii IHEM 14354 (AJ888437)
Pseudallescheria ellipsoidea FMR 8623 (AJ888427)
Pseudallescheria boydii FMR 8539 (AJ888411)
Pseudallescheria boydii FMR 7885 (AJ888392)
Pseudallescheria boydii FMR 4167 (AJ888385)
Pseudallescheria boydii FMR 7884 (AJ888391)
Pseudallescheria boydii IHEM 14451 (AJ888394)
Pseudallescheria boydii IHEM 14263 (AJ888436)
Pseudallescheria boydii FMR 8620 (AJ888417)
Pseudallescheria boydii FMR 8538 (AJ888443)
Pseudallescheria boydii FMR 6697 (AJ888387)
Pseudallescheria boydii FMR 6694 (AJ888386)
Pseudallescheria boydii FMR 8537 (AJ888410)
Pseudallescheria boydii FMR 8540 (AJ888412)
Pseudallescheria boydii RV 43605 (AJ888383)
Pseudallescheria boydii IHEM 14358 (AJ888438)
Pseudallescheria boydii IHEM 14638 (AJ888398)
Pseudallescheria boydii CBS 101.22 (AJ888435)
Pseudallescheria boydii MUCL 14009 (AJ888422)
Pseudallescheria ellipsoidea CBS 418.73 (AJ888426)
Pseudallescheria minutispora FMR 4072 (AJ888384)
Pseudallescheria minutispora MUCL 29258 (AJ888424)
Pseudallescheria angusta FMR 8541 (AJ888413)
Pseudallescheria angusta CBS 254.72 (AJ888414)
Scedosporium dehoogii MCC 1044 (HF570054)
Scedosporium dehoogii FMR 6921 (AJ888389)
Scedosporium dehoogii FMR 8532 (AJ888407)
Scedosporium dehoogii FMR 8622 (AJ888419)
Scedosporium dehoogii MUCL 8522 (AJ888421)
Scedosporium dehoogii MCC 1043 (HF570053)
Scedosporium dehoogii MUCL 20263 (AJ888423)
Scedosporium aurantiacum FMR 8630 (AJ888440)
Scedosporium aurantiacum IHEM 15458 (AJ888441)
Scedosporium aurantiacum IHEM 15579 (AJ888439)
Scedosporium aurantiacum RKI 2782/95 (AJ888432)
Pseudallescheria africana CBS 311.72 (AJ888425)
Scedosporium prolificans FMR 7294 (AJ888444)
85
52
51
82
61
95
88
60
89
100
96
99
0.01

PLATE. 3. Neighbor-joining tree derived from the ITS region rDNA sequences of *Scedosporium* spp. Bootstrap values were calculated from 1000 replications. Bootstrap below 50% were deleted.

India and outside Europe. Isolation of these strains from a single soil sample is an important factor as there are growing numbers of immunocompromised patients in Pune, India due to immunopulmonary infections (Salvi & Barnes 2009), AIDS, H1N1, and other viral infections caused by compromised hygiene in many hospitals. Although Gilgado et al. (2009) has shown that *S. dehoogii* is pathogenic to immunocompetent mice, detailed surveys of different natural and human dominated habitats are required to confirm distribution of *Scedosporium* in India as noted by Kaltseis et al. (2009) for Austria and the Netherlands. Research on secondary fungal infections of immunocompromised patients may also determine whether the victims are actually succumbing to secondary infection caused by *Scedosporium* or similar fungi.

Acknowledgments

The authors thank DBT, India for funding and Director, NCCS, India for laboratory facilities. Dr. S.V. Shinde, University of Pune, India is acknowledged for SEM microscopy. Authors also thank Dr. J. Guarro, Universitat Rovira i Virgili, Spain and Dr. G.S. de Hoog, CBS, Netherlands for reviewing the manuscript and suggestions.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.247

Volume 124, pp. 247–254 *April–June 2013*

Inocybe nitidiuscula and its ectomycorrhizae associated with *Alnus nitida* from Galyat, Pakistan

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Abstract — *Inocybe nitidiuscula* and its ectomycorrhizae are characterized from Pakistan morphologically and using rDNA internal transcribed spacer sequences. The sequences of above- and below-ground structures exhibit >97% similarity. This represents a new species record for Pakistan and the first description of ectomycorrhiza formed by *I. nitidiuscula*.
Keywords — alders, euagarics, Himalayan moist temperate forest, morphotype

Introduction

Inocybe (Fr.) Fr. (*Agaricales, Basidiomycota*) is a large, widely distributed genus of gilled fungi with more than 500 species worldwide (Kirk et al. 2008). This genus is characterized by a fibrillose pileus, equal or bulbous stipe, metuloids or thin walled cystidia, and yellowish brown smooth to angular-nodulose ellipsoid to amygdaliform spores. Many *Inocybe* species have thick walled pleuro-cheilocystidia (metuloids) that others lack (Heim 1931, Kühner & Romagnesi 1953, Kühner 1980, Kuyper 1986, Singer 1986, Stangl 1989, Kobayashi 2002).

Inocybe species play a very important role in the ecology of coniferous and deciduous trees by forming ectomycorrhizal associations (Larsson et al. 2009). The salient features of *Inocybe* ectomycorrhizae (EcM) are shiny ramifications, absence of rhizomorphs and cystidia, emanating hyphae with clamp connections, and a short exploration type (Agerer 2006). EcM have been reported for *Inocybe appendiculata* Kühner, *I. avellana* P. Kumm., *I. fuscomarginata* Kühner, *I. heimii* Bon, *I. lacera* (Fr.) P. Kumm., *I. lanuginella* (J. Schröt.) Konrad & Maubl., *I. obscurobadia* (J. Favre) Grund & D.E. Stuntz, *I. petiginosa* (Fr.) Gillet, and *I. terrigena* (Fr.) Kuyper. So far, EcM have not been described for *I. nitidiuscula* with any host plant.

Galyat, a narrow 250,000 acre strip of Himalayan moist temperate forest (HMTF) northeast of Islamabad extending on both sides of KPK-Punjab border,

is considered the best representative of HMTF with a high species diversity (Jamal & Khadija 2009, Irshad & Khan 2012). The region is characterized by mixed vegetation of deciduous and coniferous trees. Niazi (2008) and Niazi et al. (2006, 2007, 2009, 2010) have reported on previous studies on ectomycorrhizal fungi from this area.

During fieldwork in Koza Gali and Jhika Gali in Galyat, *Inocybe nitidiuscula* was collected along with belowground ectomycorrhizae associated with *Alnus nitida*. Anatomical and molecular characterization of both above- and belowground parts determined its distribution with coniferous and deciduous vegetation.

Materials & methods

Basidiomata were collected, photographed and dried in the field. Collected material was characterized anatomically and using DNA sequence data. For microscopic observation, free hand sections were stained with Congo Red and Melzer's reagent. Drawings were made using a camera lucida attached to a compound microscope.

Soil blocks of 10 × 12 cm under selected trees were dug out and washed to isolate, characterize, and identify EcM. Morphotypes were characterized morphologically following Agerer (1987–2006) and have been deposited in the Herbarium of the Botany Department, University of the Punjab, Lahore (LAH).

DNA was extracted using the Extract-N-Amp™ Plant kit (Sigma, St Louis, MO, USA). The rDNA internal transcribed spacer (ITS) region was amplified using the primer pair ITS1F (Gardes et al. 1991) and ITS 4 (White et al. 1990) with denaturation at 94°C for 4 min, followed by 35 cycles of 45 sec at 94°C, 45 sec at 54°C, and 1 min 30 sec at 72°C, and a final extension at 72°C for 2 min. PCR products from basidiomata and EcM morphotypes were sent for bidirectional sequencing (Macrogen, Korea). Sequences were queried to GenBank using the NCBI BLAST interface for sequence comparison and identification of both basidiomata and fungi on EcM root tips. Sequence alignment and phylogenetic analysis were performed using Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). Maximum Likelihood (ML) optimization was conducted with a Jukes-Cantor substitution model and Nearest-Neighbor-Interchange (NNI) as the heuristic search method. Branch support was assessed using 1000 nonparametric bootstrap replicates. Selected sequences were aligned using Clustal W and corrected manually. The specimens are deposited in the Herbarium of the Department of Botany, University of the Punjab, Lahore (LAH) and the Herbarium of Hokkaido University Museum, Sapporo, Japan (SAPA). Nucleotide sequences are deposited in GenBank as accession numbers HE862959 (basidiome), and HF565509 (ectomycorrhizal root).

Results

Inocybe nitidiuscula and its ectomycorrhizae were identified morpho-anatomically and molecularly. The ITS sequences from basidiomata and EcM morphotypes obtained in the present study exhibited 97–99.8% sequence identity, with identification confirmed by phylogenetic analysis. In both maximum likelihood and neighbor-joining methods, all sequences of

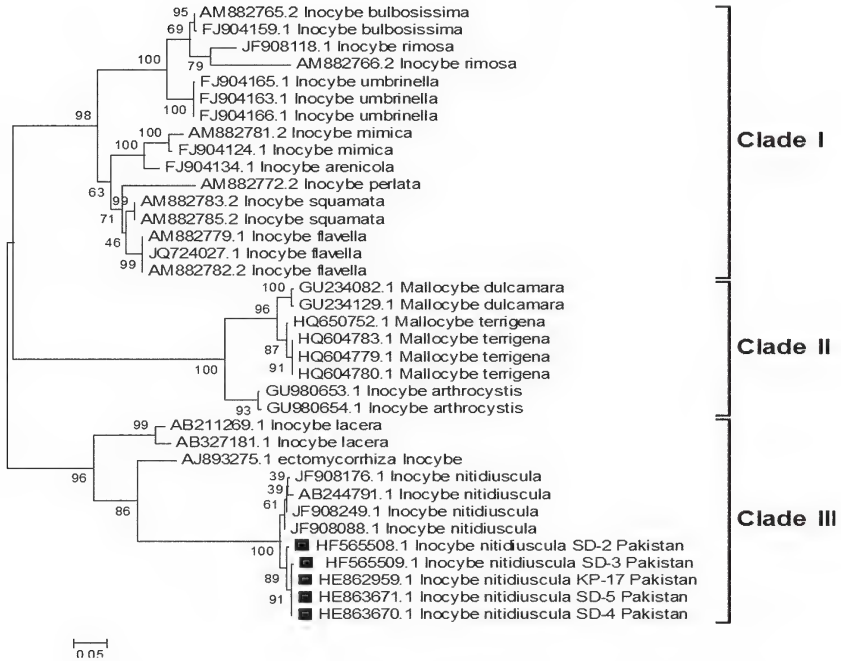

FIG. 1. Phylogenetic relationship of *I. nitidiuscula* (■) with other members of *Inocybe* based on Maximum Likelihood analysis of nrITS sequences. Bootstrap values based on 1000 replicates are shown above the branches (values <50 are not shown). The analysis included 35 sequences. All positions containing gaps and missing data were eliminated, yielding a total of 616 nucleotide positions in the final dataset. Clade I contains members of *Inocybe* sect. *Rimosae*, Clade II members of *Inocybe* subg. *Mallocybe*, and Clade III members of *Inocybe* having metuloid pleurocheilocystidia.

I. nitidiuscula from Pakistan clustered with sequences of *I. nitidiuscula* (FIG. 1, clade III) with significant bootstrap support.

Taxonomy

Inocybe nitidiuscula (Britzelm.) Lapl., Dict. Iconogr. Champ. Sup.: 523, 1894. FIG. 2

PILEUS 2.2–3.5 cm broad, light brown to dark brown from margins to centre, conical when young and plano-convex at maturity, sharply umbonate, shiny, smooth or minutely woolly at very center, umbo dark brown to blackish, outwards radially fibrous, uniformly aligned brown fibrils move toward margins which lighten in colour, margins not smooth, somewhat dentate. LAMELLAE cream to light brown, sinuate in attachment to stipe, margins dentate, lamellulae two-tiered, alternating with lamellae. STIPE 3.4–5.4 × 0.35–0.42 cm, central,

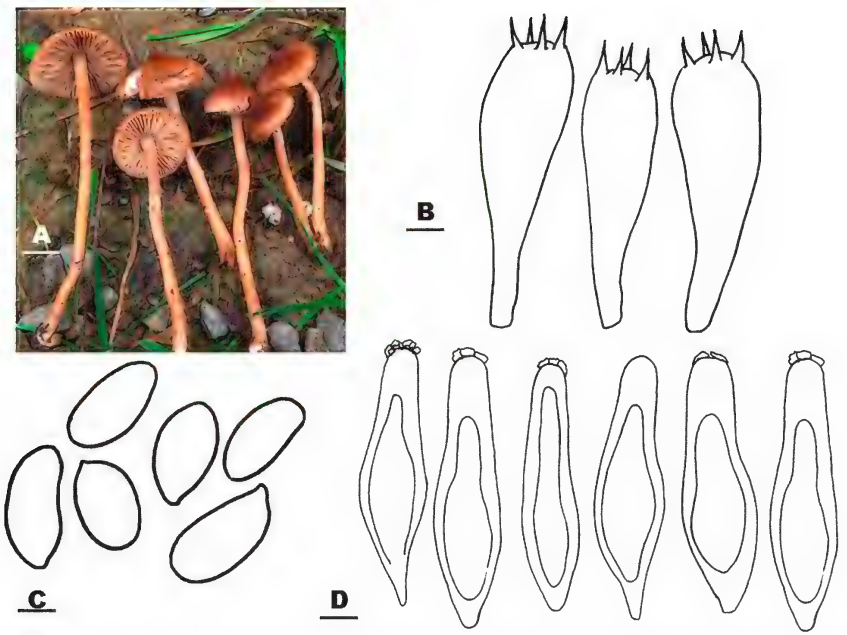

FIG. 2: *Inocybe nitidiuscula*. A. Basidiomata; B. Basidia; C. Basidiospores; D. Metuloids (cheilocystidia and pleurocystidia). Scale bars: A = 1 cm, B–C = 3.45 µm, D = 6.25 µm.

light brown, smooth, cylindrical, slightly swollen at base. ODOR not distinctive. TASTE not recorded.

Basidiospores 10–11 × 6.5–8µm, smooth, amygdaliform in side view, elliptical to ellipsoid in front view, yellowish brown, thick walled, with a prominent apiculus. BASIDIA 29–31 × 9.0–10.5 µm, tetra sterigmate, clavate, with olive green droplets in 5% KOH. PLEUROCYSTIDIA metuloids, 47.0–71.5 × 12.0–17.5 µm, abundant, cylindrical to fusiform, contents hyaline to pale yellow in 5% KOH with thick brown walls, apices heavily encrusted with colourless crystals. CHEILOCYSTIDIA similar to pleurocystidia, abundant, 38–41 × 11.8–13 µm, hyaline, clustered, thick walled, with encrusted apices. PILEIPELLIS a cutis of loosely arranged hyphae, light yellowish-brown, 7–9 µm wide. CLAMP CONNECTIONS abundant in all tissues.

MATERIAL EXAMINED: **PAKISTAN: KHYBER PAKHTUNKHWA,** Galyat, Ayubia, Koza Gali, 2250 m a.s.l., gregarious, on moist ground under *Alnus nitida*, 23 August 2010, Abdul Razaq, KP-17 (LAH 230817, SAPA 100000; GenBank, HE862959).

Morpho-anatomical description of ectomycorrhizae FIG. 3

ECTOMYCORRHIZAL SYSTEM dichotomous, axis 3–6 × 1.0–1.5 mm., in close contact with surrounding soil due to gelatinous mantle. UNRAMIFIED ENDS straight to beaded, 1–2 mm long and 0.5–1 mm in diam.; younger tips creamy

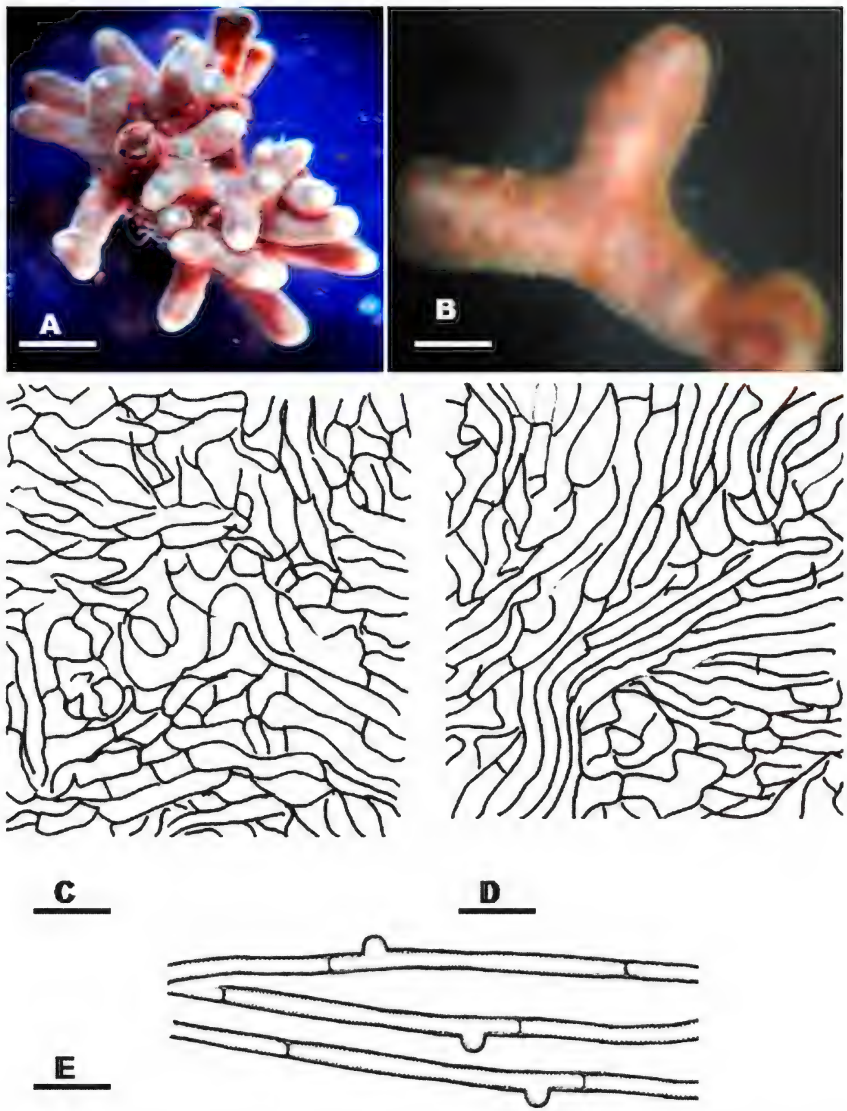

FIG. 3: *Inocybe nitidiuscula* A,B. Ectomycorrhizae. C. Outer mantle layer D. Inner mantle layer. E. Emanating hyphae. Scale bars: A = 5.4 mm, B = 3 mm, C–E = 9 µm

white, older tips light brown. Texture of system smooth with shiny luster; host tissue visible under the sheath. RHIZOMORPHS absent. EMANATING HYPHAE frequent, straight, frequently clamped; clamps large, half or more than a half

semicircle; anastomoses H-shaped. Cystidia absent, Color Reaction absent with lactic acid & KOH.

Mantle plectenchymatous in all layers. Outer Mantle loosely plectenchymatous with netlike hyphal arrangement (Agerer 1987–2006, type A), hyphae 2–3 µm diameter, lacking clamps and cystidia, transparent, no cell contents visible, anastomoses present. Inner Mantle densely plectenchymatous (Agerer 1987–2006, type A), hyphae 2–3 µm diameter, colorless to light yellowish, cell contents clear, no clamps or cystidia observed (Fig. 3).

Material examined: **PAKISTAN: Khyber Pakhtunkhwa**, Galyat (Koza Gali & Jhika Gali), roots of *Alnus nitida*, 23 August 2010, Sobia Ilyas, SD-3 (LAH; GenBank, HF565509).

Discussion

Inocybe is an integral part of almost all ectomycorrhizal communities in association with coniferous and deciduous trees from different areas of the world (Cullings et al. 2001, Yamada & Katsuya 2001, Obase 2006, Tedersoo et al. 2009, Walbert et al. 2010, Kennedy et al. 2011, Ma et al. 2012). Pakistani forests are well represented by different ectomycorrhizal fungi associated with wide host range (Niazi 2008, Niazi et al. 2006, 2007, 2009, 2010, Jabeen et al. 2012), but no published data on *Inocybe* is available even though it seems to be the most abundant group in this area among all euagarics. Basidiomata of *I. nitidiuscula* are also reported from different forests associated with *Picea*, *Pinus*, *Larix*, *Quercus*, *Corylus*, *Carpinus*, *Fagus*, *Salix*, and *Dryas* but no description of its mycorrhizae has yet been published (Kuyper 1986).

Inocybe nitidiuscula and *I. lacera* are closely related as both clustered together in phylogenetic analyses (Fig. 1, Clade III). Both species produce metuloid cystidia on lamellar face and edges and form ectomycorrhizal symbioses. The sequence (HE862959.1) from the *I. nitidiuscula* Pakistani basidiome clustered with *I. nitidiuscula* isolates from other regions of the world. The Pakistani sequence shows a 94–95% match with *I. nitidiuscula* (FN550920.1, HQ604085.1), although its morphological details are same as given by Obase et al. (2006). The genetic variation may be due to geographic distance.

The *I. nitidiuscula* ectomycorrhiza is characterized by smooth ramification, white to light brown color, lack of rhizomorphs and cystidia, prominent clamped emanating hyphae, and a plectenchymatous mantle. These resemble the ectomycorrhizae of *I. appendiculata* associated with *Picea* and *Abies* and *I. fuscomarginata* associated with *Salix* spp., except that the ramification type more closely resembles *I. terrigena,* reported with *Pinus* (Agerer 1987–2006).

Phylogenetic analyses are a useful tool for identifying unknown *Inocybe* sequences to the species level (Ryberg et al. 2008). Both above- and below-ground parts of the Pakistani collections cluster in the *I. nitidiuscula* clade (Fig.1, Clade III). Although *Inocybe* has been reported to associate with

alders (Tedersoo et al. 2009), to our knowledge this is the first report of EcM of *I. nitidiuscula* with that host. In one study, Tedersoo et al. (2006) reported ectomycorrhiza of *Inocybe* sp. 5 (AJ893275.1) that showed maximum sequence identity with *I. nitidiuscula* (AJ534934), although with only 77% sequence similarity. In our phylogenetic analysis, this sequence clustered in the same clade as *I. nitidiuscula* but significantly distant from sequences from Pakistan, Japan, and Italy. Previous studies have shown that alder ectomycorrhizal communities tend to be host specific (Rochet et al. 2011). Tedersoo et al. (2009) indicated that ascomycete EcM symbionts of *Alnus* might not be host-limited, although their results suggested high *Alnus* specificity in basidiomycete symbionts. A detailed study on *Alnus* ECM communities from Pakistan will determine the level of host specificity.

Acknowledgements

We are thankful to Dr. Abdul Rehman Khan Niazi and Dr. Todd Osmundson for acting as expert reviewers to improve the manuscript and Dr. Takahito Kobayashi, Curator of SAPA Herbarium, Japan, for the identification of specimens. Higher Education Commission, Pakistan, provided financial support for this research work.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.255

Volume 124, pp. 255–262 *April–June 2013*

Xerocomus porophyllus sp. nov., morphologically intermediate between *Phylloporus* and *Xerocomus*

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Abstract— *Xerocomus porophyllus*, discovered from Dinghushan Biosphere Reserve in Guangdong province (southern China), is described as a new species. It is a morphologically intermediate taxon between *Phylloporus* and *Xerocomus* since its hymenophore is lamellate around the stipe but strongly anastomosing to distinctly faveolate or poroid towards pileus margin. Molecular analyses of nuclear rDNA ITS and LSU sequences, however, indicate that it has a closer genetic relationship with *Xerocomus* than with *Phylloporus*.

Key words— *Basidiomycota*, *Boletaceae*, *Boletales*, phylogeny, taxonomy

Introduction

Phylloporus Quél. and *Xerocomus* Quél. belong to the family *Boletaceae* and have a close relationship, whether *Phylloporus* has been considered as a primitive group in the family (Corner 1972) or as a derived genus next to *Xerocomus* (Binder 1999; Binder & Hibbett 2006). Molecular data have shown the *Xerocomus subtomentosus* complex to be closely related to *Phylloporus* (Binder 1999). The lamellate hymenophore in *Phylloporus* and the wide poroid or tubular one in *Xerocomus* are still used as the main morphological characters for differentiating the two genera (Neves & Halling 2010; Neves et al. 2012).

Worldwide, *Phylloporus* encompasses about 70 named species (Neves et al. 2012), 21 of which have been reported from China (Teng 1963; Zang & Zeng 1978; Li et al. 1992; Bi et al. 1993, 1997; Dai & Li 1994; Zang et al. 1996; Zeng et al. 2012). However, two Chinese records should be excluded because the type specimen of *Phylloporus scabrosus* M. Zang is a *Lentinus* sp., and Chinese exsiccatae labelled as "*Phylloporus rhodoxanthus*" were misidentified (Zeng et al. 2012).

Although some mycologists have incorporated *Xerocomus* into *Boletus* (Corner 1972, Kirk et al. 2008), other authors still treat it as an independent

genus (Watling & Hills 2005, Hills 2008, Neves et al. 2012, Zeng et al. 2012). Until now, more than 210 species and varieties of *Xerocomus* have been registered (www.indexfungorum.org), of which at least 46 species have been reported from China (Zhang et al. 2012).

Recently, an interesting bolete was recorded in Dinghushan Biosphere Reserve in Guangdong province (southern China) and found to differ from all known taxa. Although its hymenophore is consistently lamellate (*Phylloporus*-like) around the stipe, it is also obviously faveolate or tubular (*Xerocomus*-like) towards the pileal margin. Molecular analyses of nuclear rDNA ITS and LSU sequences, however, indicated it to have a closer genetic relationship with *Xerocomus* than with *Phylloporus*. Further investigation revealed that it is a new species to science, and therefore it is formally described here. The holotype is deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM).

Materials & methods

Specimens were annotated and photographed in the field, and dried in an electric drier. Color descriptions were made according to Kornerup & Wanscher (1978). Tissue sections were mounted in 5% KOH or 1% Congo Red for microscopic examination. Dimensions of basidiospores are given using the notation (a–)b–c(–d), where 'b–c' represents 90% or more of the measured values of at least 30 basidiospores, and the parenthetical 'a' and 'd' are the extreme values. Lm refers to the average length of all basidiospores standard deviation; Wm refers to the average width of all basidiospores standard deviation; Qm refers to the average Q of all basidiospores standard deviation; and n refers to the number of spores measured per specimen. Basidiospores were also observed under a scanning electron microscope (SEM). Light micrographs were taken using an Olympus BX51 trinocular phase contrast microscope and the scanning electron micrographs were taken on a Philips FEI-XL30 scanning electron microscope.

Genomic DNA was isolated from dried specimens and the ITS1-5.8S-ITS4 segment from the ribosomal DNA (rDNA) was amplified with primer sets and ITS1 (5′-CTT GCG TTG ATT ACG-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) by polymerase chain reaction (PCR) techniques (White et al. 1990). A portion of the nuclear ribosomal large subunit (nrLSU) was amplified with the primers LR6 (5′-CGC CAG TTC TGC TTA CC-3′) and LROR (5′-ACC CGC TGA ACT TAA GC-3′) (Vilgalys & Hester1990). Amplified products were examined with agarose gel electrophoresis using a 2kb DNA marker. The amplified fragments were cloned into pCR2.1 TOPO (Invitrogen) to generate, the vector pCR2.1 TOPO containing the targeting fragment was transformed into its host *Escherichia coli* strain JM105 (López-García et al. 2003). The *E. coli* with the cloned fragments was sequenced by BGI Company and the data were deposited in GenBank. For the analysis of phylogenetic placement of the new species, the rDNA sequences of other related *Phylloporus* and *Xerocomus* taxa were downloaded from Genbank. Phylogenetic tree inferred from LSU rDNA sequences was constructed using maximum likelihood (ML) in Mega 5.05. Bootstrap analysis was implemented with 1000 replicates.

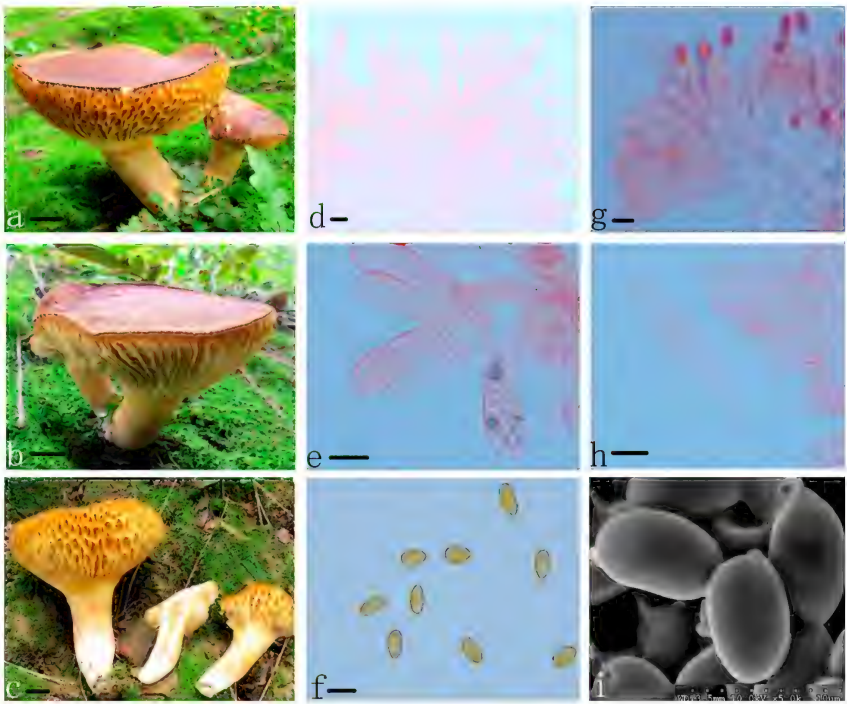

Fig. 1. *Xerocomus porophyllus* (GDGM 30303, holotype): a–c. basidiomes; d. pileipellis; e. basidia; f. basidiospores; g. hymenium with basidia, pleurocystidia and basidiospores; h. pleurocystidium; i. basidiospores under SEM. Scale bars: a–c = 1 cm; d–h = 10 μm.

Taxonomy

Xerocomus porophyllus T.H. Li, W.J. Yan & Ming Zhang, **sp. nov.** Figs. 1–2

MycoBank **MB 563347**

Differs from *Phylloporus cingulatus* by its larger basidiospores and non-cyanescent context on exposure; and from all species of *Xerocomus* s. str. by the largely lamellate hymenophore near the stipe.

Type: China, Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, 23°10.11′N 112°31.50′E, alt. 300 m a.s.l., 17 May 2012, Tai-Hui Li, Wen-Juan Yan & Hao Huang (**Holotype**, GDGM 30303; GenBank KC168089, KC561775).

Etymology: *porophyllus* refers to the partially poroid and partially lamellate hymenophore of the new species.

Pileus 50–80 mm broad, fleshy, convex, becoming plane to revolute with age, dry, even, subtomentose, matted subtomentose to slightly scurfy, often faintly and finely rimulose; grayish-ruby (12D5–6, 12E5). Flesh 5–20 mm thick at stipe, white to buff, sometimes with a very faint pinkish tint, unchanging

when injured, with mild odor and flavor. Hymenophore brownish-orange (5C4–6, 6C5–6), 2–5 mm deep, decurrent, lamellate near the stipe and strongly anastomosing, poroid or faveolate towards pileus margin, with 48–76 lamellae; pores 1.5–3 mm wide near pileus margin. Stipe cylindrical, 33–45 mm long, 5–15 mm wide at apex, attenuated at the base, dry, pale yellow to light yellow (4A3–4), solid when young, becoming hollow with age, with white basal mycelium; stipe context white (1A1), unchanging or with pale pinkish flush when exposed.

Basidiospores (5.5–)7.5–10.5(–12.8) × (4.0–)5.0–5.8(–7.0) µm [n = 30, Lm = 9.57 ± 1.17, Wm = 5.15 ± 0.86, Qm = 1.79 ± 1.41], ellipsoid, smooth under light microscope but nearly smooth to finely rugulose or weakly bumpy under SEM, yellow to olivaceous-yellow in KOH, inamyloid in Melzer's reagent. Basidia clavate, 28–34 × 8.0–11 µm, 2–4-spored, hyaline; sterigmata (2–) 3–5(–8) µm long. Pleurocystidia 42–68 × 9.0–12.5 µm, fusiform, thin-walled, hyaline. Caulocystidia and Cheilocystidia absent. Hymenophoral trama subparallel to nearly bilateral with hyphae of the lateral strata loosely arranged, hyaline, 4.5–19 µm broad. Pileipellis composed of tufts of erect hyphal elements 4–21 µm broad, terminal cells 11–55 × 4.5–20 µm, clavate, brownish-yellow to yellowish. Clamp connections absent in all tissues.

Ecology and Distribution — Solitary or clustered on soil under *Schima superba* Gardner & Champ. (*Theaceae*) in a mixed forest with *Pinus massoniana* Lamb. (*Pinaceae*) and other frondose trees. May to June. Known only from the type locality in southern China (Guangdong).

Comments — The new species is considered morphologically intermediate between *Phylloporus* and *Xerocomus* because of its dominantly lamellate hymenophore near the stipe, with the strongly anastomosing gills forming a faveolate or poroid pattern towards the pileus margin. The nearly smooth to finely rugulose spores observed under SEM are quite common in both *Phylloporus* and *Xerocomus subtomentosus* complex (Šutara 2008, Neves & Halling 2010, Zeng et al. 2012), which also implies that the two genera are closely related.

Many species of *Phylloporus* have a partially poroid hymenophore. The species most similar to *X. porophyllus* is *P. cingulatus* Corner with strongly anastomosing lamellae or poroid hymenophore, but differing in the slowly cyanescent context on exposure and the smaller spores (7.5–9 × 5–5.5 µm; Corner 1970). Among other species of *Phylloporus* with strongly anastomosing lamellae or poroid hymenophore are: *P. albocarnosus* Heinem., *P. ater* (Beeli) Heinem., *P. boletinoides* A.H. Sm. & Thiers, *P. carmineus* Heinem., *P. fibulatus* Singer et al., *P. ochraceobrunneus* Corner, *P. pumilus* M.A. Neves & Halling, *P. purpureus* var. *ambiguus* Heinem., and *P. testaceus* Heinem. & Gooss.-Font., which can all be easily distinguished from *X. porophyllus* by their pileus colors

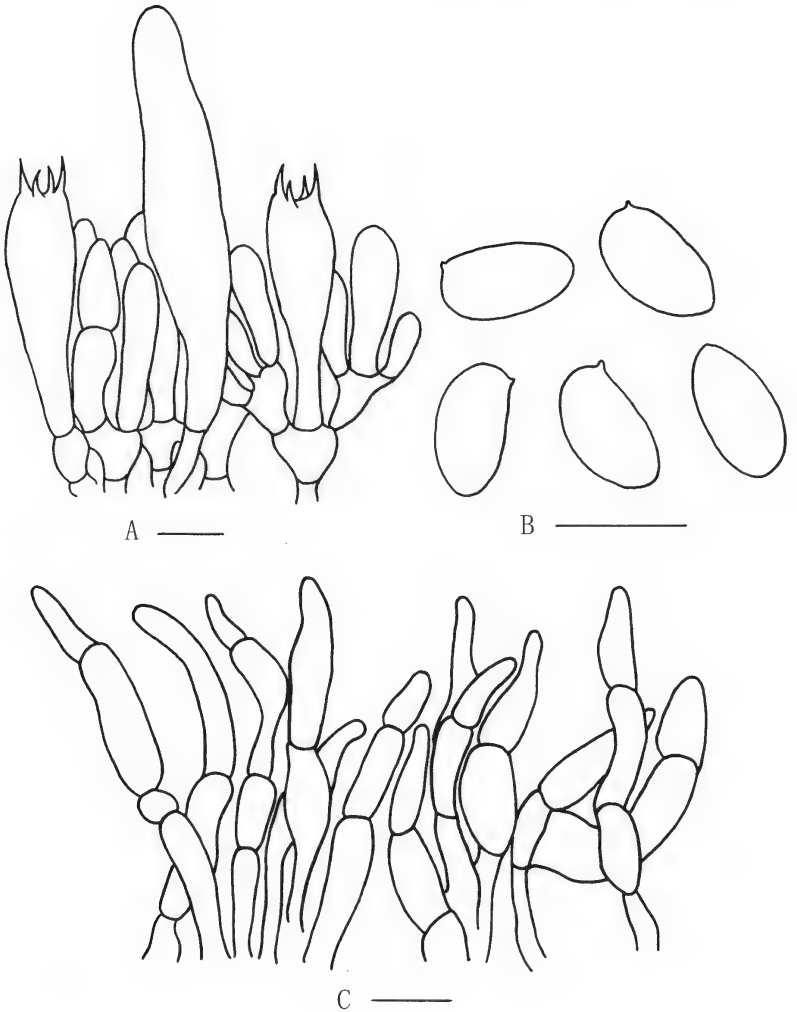

FIG. 2. *Xerocomus porophyllus* (GDGM 30303, holotype): A. basidiomes; B. basidiospores; C. pileipellis. Scale bars: A–B = 10 μm; C = 20 μm.

and sizes of basidiospores or basidiomes. *Phylloporus albocarnosus* has a white to straw-colored pileus and smaller spores (7–8 × 3–4 μm); *P. ater* is a blackish species; *P. boletinoides* shows a color variability ranging from cinnamon-brown or dark vinaceous-brown to cocoa-brown; *P. carmineus* is a carmine-red species; *P. fibulatus* has a much smaller (11–35 mm) and yellow to dull yellow pileus; *P. ochraceobrunneus* has fuscous-brown to brownish-yellow pileus and

Table 1. Sequence identity comparisons of *Xerocomus porophyllus* with species of *Xerocomus* and *Phylloporus*.

Species	Sequence	BLAST max score
ITS		
Xerocomus sp.	FJ197009.1	695 (88% query coverage) (85% max identity)
Xerocomus sp.	EU569235.1	695 (88% query coverage) (85% max identity)
Boletus dryophilus (≡ *Xerocomus dryophilus*)	AY185181.1	691
Boletus dryophilus (≡ *Xerocomus dryophilus*)	AY185183.1	686
Phylloporus sp.	JQ003620.1	368
Phylloporus sp.	JQ003650.1	359
Phylloporus rhodoxanthus	DQ533980.1	346
Phylloporus foliiporus	JQ003641.1	344
LSU		
Xerocomus fennicus	AF514820.1	1127 (100% query coverage) (94% max identity)
Xerocomus riparielus	AF514818.1	1127 (100% query coverage) (94% max identity)
Phylloporus centroamericanus	JQ003663.1	1075
Phylloporus purpurellus	JQ003672.1	1016

larger spores (11–15 × 4–4.7 µm); *P. pumilus* has a very small (5–9 mm) cocoa brown or dark-brown pileus; *P. purpureus* var. *ambiguus* has a carmine-purple pileus and larger spores (9.3–12 × 4.2–5.3 µm); and *P. testaceus* has a rose-ochre to deep carmine-brown pileus and much larger spores (11.5–14.0 × 5.0–6.0 µm) (Heinemann 1951, 1955, Corner 1970, Singer et al. 1990, Neves & Halling 2010, Neves et al. 2012). Although some species of *Xerocomus*, such as *X. armeniacus* (Quél.) Quél. (≡ *Xerocomellus armeniacus* (Quél.) Šutara) and *X. chrysenteron* (Bull.) Quél. (≡ *Xerocomellus chrysenteron* (Bull.) Šutara), may have a subtomentose and finely rimulose pileus with similar color as *X. porophyllus*, their entirely poroid hymenophore is easily differentiated from the largely lamellate pattern of the latter.

The low max identities of the sequences demonstrate that the species compared are obviously different, and the higher max scores with the *Xerocomus* species also indicate that *X. porophyllus* has a closer genetic relationship with *Xerocomus* than with *Phylloporus* (Table 1). In a phylogenetic tree constructed with the LSU sequences of the new species, eight *Phylloporus* species and eight other *Xerocomus* species, with *Auricularia auricula-judae* (Bull.) Quél. as outgroup, *X. porophyllus* is grouped with the other *Xerocomus* species (Fig. 3).

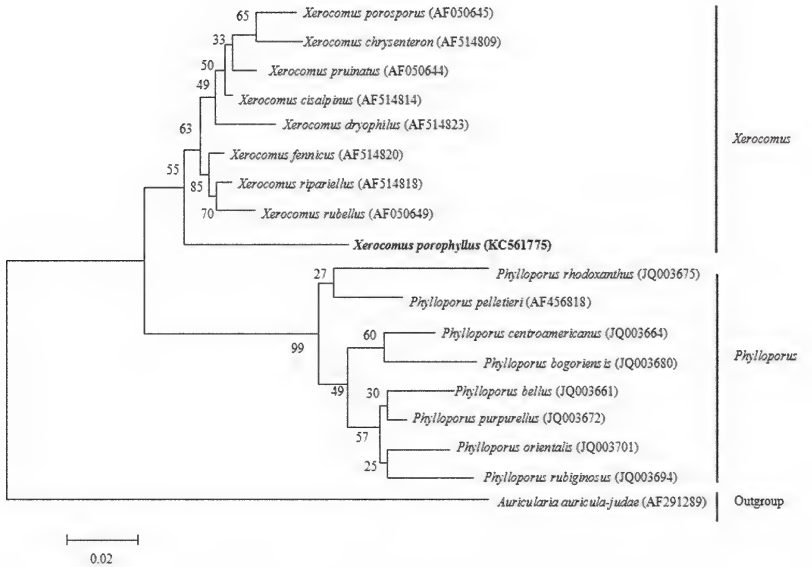

FIG.3. Phylogenetic placement of *Xerocomus porophyllus* inferred from LSU rDNA data. The bootstrap values (1000 replicates) are shown next to the branches. Taxa are labeled by their names and GenBank accession numbers.

Therefore, the novel entity is described as a new species of *Xerocomus* according to morphological and molecular analyses.

Acknowledgments

The authors are very grateful to Dr. Beatriz Ortiz-Santana (US-Forest Service, Northern Research Station, Center for Forest Mycology Research, US) and Dr. Matteo Gelardi for reviewing the manuscript. The study is supported by the National Natural Science Foundation of China (No. 30970023, 31070024, 30870019) and the Natural Science Foundation of Guangdong Academy of Sciences for Young Scholars, China (No. qnjj201202), Science and Technology Planning Project of Guangdong Province, China (No. 2012B091100258, 2012B091100072).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.253

Volume 124, pp. 263–268 *April–June 2013*

Glomus mume and *Kuklospora spinosa*: two new species of *Glomeromycota* from China

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Abstract — Two new arbuscular mycorrhizal fungal species, *Glomus mume* (*Glomerales*) and *Kuklospora spinosa* (*Diversisporales*), were isolated from rhizosphere soil of *Prunus mume* in China. *Glomus mume* resembles *G. taiwanense*, but differs by its spore walls swelling to a globose to subglobose structure in or near the middle of the spore apex. *Kuklospora spinosa* resembles *K. colombiana* but differs by the fine spines covering the middle-layer of the outer spore wall.

Key words —morphotaxonomy, Guizhou, Szechwan, HMAS

Introduction

During a survey of arbuscular mycorrhizal fungi associated with *Prunus mume* (Siebold) Siebold & Zucc. (*Rosaceae*) in China, spores of an undescribed species of *Glomus* Tul. & C. Tul. were found in the soil samples collected from the Maolan National Nature Reserve (25°14′–25°18′N 107°56′–108°10′E) of subtropical woodland in southeast Guizhou province. A second undescribed species, representing *Kuklospora* Oehl & Sieverd., was found in the soil samples collected from the brushy mountain of Muli county (27°58′N 101°15′E) in Szechwan province. The two arbuscular mycorrhizal fungi are described herein as *Glomus mume* and *Kuklospora spinosa*, based on spore morphology.

Materials & methods

Soils were collected from the rhizosphere of *Prunus mume*, from 5–20 cm soil depth. The soil samples of ca. 1000 g were placed in sterilized cotton-cloth bags, labeled, and

† Prof. Jun-yu Chen, a senior member of Chinese Academy of Engineering, died on 8 June 2012.

air-dried in shade. Then they were cleaned from plant debris, sieved with a 2 mm sieve, stored at 4°C, until processed.

Spores were isolated according to Gerdemann & Nicolson (1963) with the wet sieving and decanting method as modified by Daniels & Skipper (1982). Arbuscular mycorrhizal fungal spores were identified following the descriptions of Schenck & Pérez (1988), Morton & Redecker (2001), and more recent species descriptions of *Glomeromycota*. We follow the classification proposed and summarized by Oehl et al (2011). Colors were referred to http://invam.caf.wvu.edu/otherinfo/articles/colorchart.jpg, the INVAM color chart. Specimens were deposited at the Herbarium Mycologicum Academiae Sinicae (HMAS) in Beijing.

Taxonomy

Glomus mume B.P. Cai, Jun Y. Chen, Q.X. Zhang & L.D. Guo, **sp. nov.** Plate 1:A–C

Fungal Name 570036

Differs from *Glomus taiwanense* by its spore walls swelling to a globose to subglobose structure, in or near the middle of the spore apex.

Type: China, Guizhou, Libo, Maolan National Nature Reserve, from the rhizosphere of *Prunus mume*, 17 Sep. 2005 (**Holotype**, HMAS143348).

Etymology: Referring to the plant species with which this fungus was originally associated.

Sporocarps yellow-brown (20–20–20–0) to dark brown (0–30–40–0), globose to subglobose, 350–600 × 380–650 µm, with spores formed radially in a single, tightly packed layer around a central plexus of hyphae. Peridium absent. Spores clavate, cylindro-clavate, triangular or irregular, 90–165 × 30–50 µm, tapering to a cylindric subtending hypha 6–12 µm. Chlamydospore wall laminate, yellow-brown (20–20–20–0) to dark brown (0–30–40–0), 1.5–4 µm thick on the side walls, swelling to a globose to subglobose structure, 13–20 × 15–25 µm, in or near the middle of the spore apex, and thickened to 3–7 µm at spore base. Spore base at hyphal attachment with a small pore, open or occluded by wall thickening. Reaction to Melzer's reagent not distinctive.

Ecology — Spores of *Glomus mume* were isolated from soil sampled from the rhizosphere of *Prunus mume* growing at 700–800 m a.s.l in the karst forest area of southwest China (25°19′N 107°57′E). The chemical properties of the soil sample were: pH 6.62, organic matter 146.27 $g.kg^{-1}$, total N 6.5 $g.kg^{-1}$, organic N 0.51 $g.kg^{-1}$, total P 0.48 $g.kg^{-1}$, organic P 0.025 $g.kg^{-1}$, and exchangeable K 0.13 $g.kg^{-1}$.

Glomeralean species occurring with *G. mume* were *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler, *Funneliformis geosporus* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, *Glomus australe* (Berk.) S.M. Berch, *G. clavisporum* (Trappe) R.T. Almeida & N.C. Schenck, *G. multicaule* Gerd. & B.K. Bakshi, *G. rubiforme* (Gerd. & Trappe) R.T. Almeida & N.C. Schenck, and *Septoglomus deserticola* (Trappe et al.) G.A. Silva et al.

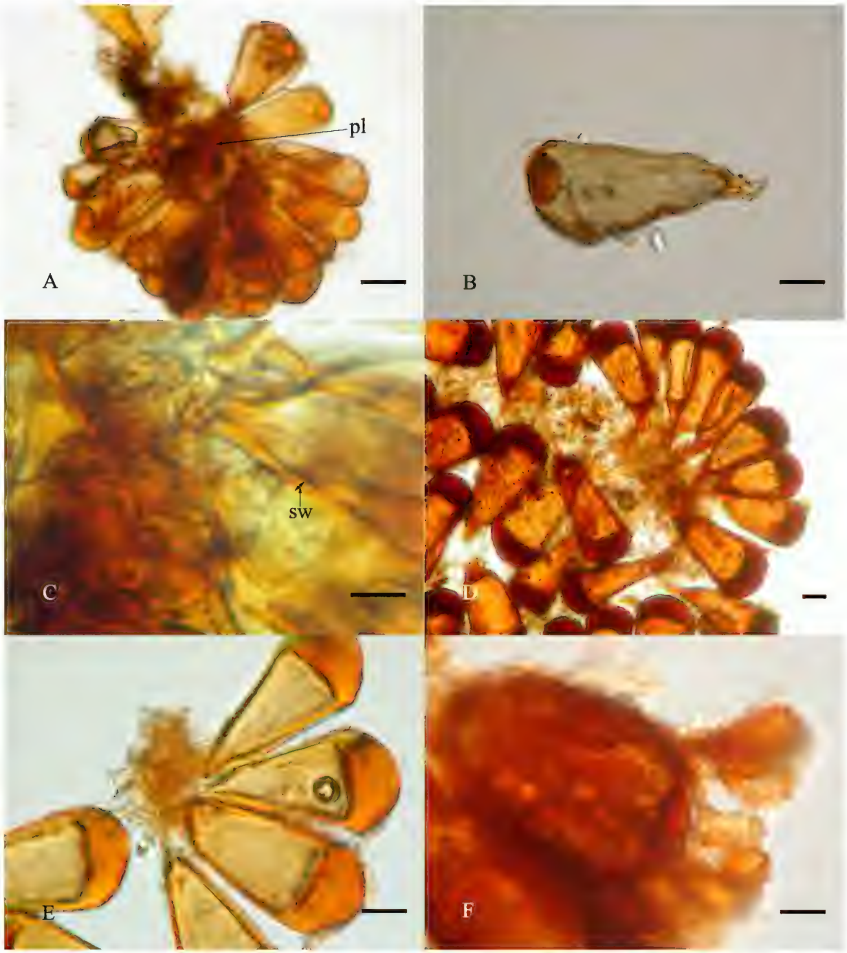

Plate 1. *Glomus mume* and morphologically similar species. ***Glomus mume***: A. Broken sporocarp showing the central hyphal plexus (pl) in Melzer's reagent; B. Spore in Melzer's reagent; C. Structure of the spore wall (sw) in Melzer's reagent. ***Glomus clavisporum***: D. Portion of the sporocarp. ***Glomus taiwanense*** E. Portion of the sporocarp. ***Glomus liquidambaris***: F. Spores with "paraphysis-like" structures. Scale bars: A, B, D, E, F = 20 µm; C = 10 µm.

Kuklospora spinosa B.P. Cai, Jun Y. Chen, Q.X. Zhang & L.D. Guo **sp. nov.** Plate 2

Fungal Name 570037

Differs from *Kuklospora colombiana* by fine spines covering the middle-layer of the outer spore wall.

Type: China, Szechwan, Muli country, from the rhizosphere of *Prunus mume,* 5 Sep. 2005 (**Holotype**, HMAS142950).

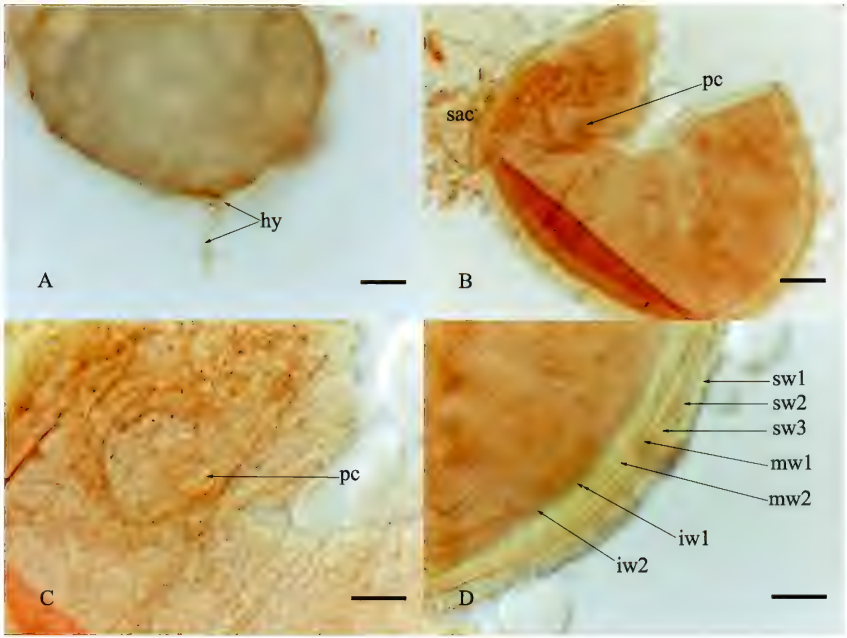

Plate 2. *Kuklospora spinosa*. A. Spore with a distal hypha (hy) in water; B. Spore connections to the sporiferous saccule (sac) and the cicatrix proximal to saccule (PC) in Melzer's reagent; C. Cicatrix proximal to saccule (PC) in Melzer's reagent; D. Broken spore with three-layered outer wall (sw1–3), two layers of the middle wall (mw1 & mw2) and the inner wall (iw1 & iw2); iw2 stained by Melzer's reagent. Scale bars: A, B = 20 µm; C, D = 10 µm.

Etymology: Latin, *spinosa*, referring to the ornamentation on the surface of the outer laminate spore wall layer (sw2).

Sporocarps unknown. Spores formed by inflating the hyphal stalk in some distance to a terminally or intercalary formed sporiferous saccule. Spores hyaline, subhyaline to yellowish-white (0–10–20–0), globose to subglobose, 100–250 µm diam. Spores have three walls: an outer spore wall (sw), a middle wall (mw) and an inner wall (iw). The outer wall (sw) tri-layered (sw1, sw2 and sw3): sw1 hyaline, 1–1.5 µm thick, evanescent, usually completely degraded or sloughed in mature spores; sw2 laminate, yellowish-white (0–10–20–0), 3–5 µm thick, covered with fine spines, 2–3 high × 0.5 µm in diam, which grow into sw1; sw3 hyaline, 0.5–1 µm thick, tightly adherent to sw2, usually separated from sw2 in Melzer's reagent, reaction to Melzer's reagent not distinctive. The middle wall (mw) hyaline, bilayered (mw1 and mw2), thin, less than 1 µm thick, no reaction in Melzer's reagent. The inner wall (iw) bilayered (iw1 and iw2): iw1 membranous, granular, beaded, 0.5–1 µm thick, granules sometimes scattering in crushed spores; iw2 plastic, 0.5–1 µm thick, staining pink (0–30–20–0) to

reddish purple (0–40–60–10) in Melzer's reagent. Sporiferous saccule hyaline, globose, 90–150 µm diam, collapsing at maturity and leaving a cicatrix proximal to saccule and a distal hypha or cicatrix. Cicatrix proximal to saccule, 8–15 µm diam, distal hypha, 1.5–3 µm wide at the spore base. Wall of soporiferous saccule bilayered, 1.5–3 µm thick.

Ecology — Spores of *Kuklospora spinosa* were isolated from soil sampled under *Prunus mume* growing at 2450–3100 m a.s.l. in the brushy mountain of southwest China (27°58′N 101°15′E), The chemical properties of the soil sample from which *G. mume* was isolated were: pH 6.48, organic matter 56.32 $g.kg^{-1}$, total N 2.5 $g.kg^{-1}$, organic N 0.15 $g.kg^{-1}$, total P 0.48 $g.kg^{-1}$, organic P 0.037 $g.kg^{-1}$, and exchangeable K 0.2 $g.kg^{-1}$.

Glomeralean species occurring in the same samples were *Acaulospora tuberculata* Janos & Trappe, *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler, *C. luteum* (L.J. Kenn. et al.) C. Walker & A. Schüssler, *Diversispora trimurales* (Koske & Halvorson) C. Walker & A. Schüssler, *Funneliformis geosporus*, *F. mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, *Glomus clavisporum*, and *Septoglomus deserticola.*

Discussion

Glomus mume is morphologically similar to *G. clavisporum*, *G. taiwanense* (C.G. Wu & Z.C. Chen) Y.J. Yao, *G. liquidambaris* (C.G. Wu & Z.C. Chen) Y.J. Yao, and *G. coremioides* (Berk. & Broome) D. Redecker & J.B. Morton. The sporocarpic spores of *G. mume* can be easily separated from these four species by the swelling in the middle of the spore apex (Plate 1A, B). The spore wall of *G. clavisporum* is 1–1.5 µm thick at the sides, thickened to 17–25 µm at the apex and to 5–8 µm at the base (Wu & Chen, 1987; Almeida & Schenck, 1990; Plate 1D). Spore wall of *G. taiwanense* is 1–1.5 µm thick at the sides, thickens to 4–25 µm at the apex and to 1–5.5 µm at the base (Wu & Chen, 1987; Almeida & Schenck, 1990; Plate 1E). Spore wall of *G. liquidambaris* is 2–5 µm thick at the sides, thickens to 7.5–25 µm at the apex and to 6–10 µm at the base, and the prominent characteristic of this latter species is a "paraphysis-like" structure protruding from the central plexus of hyphae to the outer part of the sporocarp (Wu & Chen, 1987; Almeida & Schenck, 1990; Plate 1F). Spore wall of G. *coremioides* is 2–2.5 µm thick, up to 4 µm thick near the spore base (Almeida & Schenck, 1990).

Kuklospora was erected by Sieverding & Oehl (2006), to accommodate two species previously described in *Entrophospora: K. colombiana* (Spain & N.C. Schenck) Oehl & Sieverd. and *K. kentinensis* (C.G. Wu & Y.S. Liu) Oehl & Sieverd. *Kuklospora spinosa* has a morphology typical for the genus based on the formation of spores within the stalk of the sporiferous saccule and the presence of a beaded inner wall layer (iw1). Spores of *K. spinosa* differ clearly

from those of *K. colombiana* and *K. kentinensis* in size, colour, and outer spore wall ornamentation.

Acknowledgments

The authors acknowledge the help of Dr. Tiezheng Wei and Yi-Jian Yao, Systematic Mycology & Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. We especially thank Dr. Ewald Sieverding (Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany), Prof. Dr. Janusz Błaszkowski (Department of Plant Pathology, Academy of Agriculture, Szczecin, Poland) and Prof. Dr. Runjin Liu (Institute of Mycorrhizal Biotechnology, Qingdao Agricultural University, Shandong, China), for their valuable comments and revisions on the manuscript. We appreciate the corrections by Shaun Pennycook, Nomenclatural Editor, and suggestions by Lorelei L. Norvell, Editor-in-Chief. This study was supported in part by the National Natural Science Foundation of China Grants 30470006, the science and technology item of Xiamen (3502Z20072010 and 3502Z20102004).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.269

Volume 124, pp. 269–278 *April–June 2013*

Russula changbaiensis sp. nov. from northeast China

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Abstract —*Russula changbaiensis* (subg. *Tenellula* sect. *Rhodellinae*) from the Changbai Mountains, northeast China, is described as a new species. It is characterized by the red tinged pileus, slightly yellowing context, small basidia, short pleurocystidia, septate dermatocystidia with crystal contents, and a coniferous habitat. The phylogenetic trees based on ITS1-5.8S-ITS2 rDNA sequences fully support the establishment of the new species.

Key words —*Russulales*, *Russulaceae*, taxonomy, morphology, *Basidiomycota*

Introduction

The worldwide genus of *Russula* Pers. (*Russulaceae*, *Russulales*) is characterized by colorful fragile pileus, amyloid warty spores, abundant sphaerocysts in a heteromerous trama, and absence of latex (Romagnesi 1967, 1985; Singer 1986; Sarnari 1998, 2005). As a group of ectomycorrhizal fungi, it includes a large number of edible and medicinal species (Li et al. 2010). The genus has been extensively investigated with a long, rich and intensive taxonomic history in Europe (Miller & Buyck 2002). Although *Russula* species have been consumed in China as edible and medicinal use for a long time, their taxonomy has been overlooked (Li & Wen 2009, Li 2013). Only 14 new species and three new varieties have been reported from China (Singer 1935; Chiu 1945; Ying 1983, 1989; Bi & Li 1986; Zang & Yuan 1999; Wen & Ying 2001; Wang et al. 2009; Li et al. 2011, 2012), and a systematic investigation of the *Russulaceae* in China has also been limited until recently (Wang & Liu 2009, 2010; Das et al. 2010; Buyck & Atri 2011; Das & Verbeken 2011; Wang et al. 2012).

The Changbai Mountain range on the borders of China, Korea, and Russia, is considered a region highly diverse in macro-fungi (Xie et al. 1986). During

a foray in the Changbai Mountains, several interesting *Russula* specimens were collected; after thorough examination, they are described here as a new species.

Materials & methods

Fresh specimens were photographed using a Sony Cyber Shot DSC-S75 digital camera, and macroscopic characteristics of only the intact fresh fruitbodies were recorded under sunlight in the field. After being dried at 50–60 °C in a SIGG Dorrex food dehydrator, the specimens were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS). Small samples of fresh fruit bodies were dried in silica gel for DNA extraction. Color names and codes follow Ridgway (1912). Microscopic characters were examined under a Nikon Eclipse 80i microscope according to Yang (2000), Wang et al. (2009) and Li et al. (2012). Sulfovanillin (SV) solution was used to test chemical reactions of the rehydrated specimens.

Protocols for DNA extraction, PCR, and sequencing followed those in Li et al. (2012) and references therein. Sequences of the internal transcribed spacer (ITS) region were amplified with the primer pairs ITS1/ITS4 (Gardes & Bruns 1993). PCR products were purified with the Bioteke DNA Purification Kit (Bioteke, Beijing, China). ITS regions were sequenced with the ABI 3730 DNA analyzer and ABI Bigdye 3.1 terminator cycle sequencing Kit (BGI, Beijing, China). Sequences obtained were submitted to GenBank (accession numbers are shown in Fig 3). Other sequences of representative and closely related *Russula* taxa (Eberhardt 2002; Miller & Buyck 2002; Li et al. 2011, 2012; Li 2013) were retrieved from GenBank. Assembly and editing of sequences of each region were performed with Clustal X and BioEdit (Thompson et al. 1997, Hall 1999) and were manually modulated when necessary. Some ambiguously aligned terminal sites were excluded in sequence alignment.

Based on previous phylogenetic works (Eberhardt 2002, Miller & Buyck 2002, Ryman et al. 2003, Lebel & Tonkin 2007), *Albatrellus flettii* Morse ex Pouzar and *Gloeocystidiellum aculeatum* Sheng H. Wu were chosen as outgroup taxa.

Maximum Parsimony (MP) analysis of the phylogenetic relationships among the taxa were performed in PAUP* v.4.01 (Swofford 2004). Gaps in alignment were treated as missing data, and all sites were treated unordered and unweighted. The tree bisection-reconstruction (TBR) algorithm was performed with the heuristic search option, and bootstrap analysis was conducted with 1000 replicates (Felsenstein 1985). Consistency index (CI), retention index (RI), and tree length (TL) were also calculated. Trees were displayed with Treeview 1.6.6 (Page 1996).

Taxonomy

Russula changbaiensis G.J. Li & H.A. Wen, **sp. nov.** FIGS 1-2

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Differs from *Russula puellula* by wider pileocystidia with crystal contents.

TYPE: China, Jilin Province, Antu County, Erdaobaihe, Heping Forest Farm, 43°07′N 128°54′E, alt. 1014 m, in coniferous forest dominated by *Abies nephrolepis*, 22.VII.2010, L.D. Guo, X. Sun, G.J. Li & L.J. Xie CBS20100344 (**Holotype**, HMAS262369, GenBank KC412162); CBS20100431 (**Isotype**, HMAS262394; GenBank KC412165).

Fig. 1: *Russula changbaiensis* A. Holotype HMAS 262369; B. HMAS 262355.

Etymology: named after the mountains of the type locality.

Basidiomata small to medium sized, slender and fragile. Pileus 2–6 cm in diam., first hemispherical, then convex to applanate, sometimes slightly depressed above the stipe, or even shallow infundibuliform, Dark Vinaceous (xxvii1″) to Corinthian Red (xxvii3″) at centre rarely with small patches of Pale Yellow-Orange (iii15f), glossy, Prussian Red (xxvii5″k) to Indian Red (xxvii3″k) when dry, viscous when humid; margin incurved first, straight to slightly up-curled when mature, sometimes undulate and dehiscent, slightly striate, Pale Vinaceous (xxvii1″f) to Hydrangea Pink (xxvii1″i), Deep Corinthian Red (xxvii3″i) to Ocher Red (xxvii5″b) when dry; cutis 1/2–3/4 separable from pileus edge, sometimes with small part exfoliating. Lamellae up to 5 mm height in middle, adnate to slightly subfree, rarely forked near the stipe, slightly interveined, Cream Color (xvi19′f), brittle, Naples Yellow (x19′d) when dry; edge even, narrowing towards the pileus margin, 11–13 pieces per cm in the edge; lamellulae absent. Stipe 3–5 × 1–1.5 cm, central, smooth, subclavate to cylindrical, sometimes a little ventricose toward the base, without annulus, White (liii), partly turning Baryta Yellow (iv21f) when aged and dry, rarely with small Cinnamon (xxix15″) spots at the base, not pruinose, fragile, hollow when mature. Context 2–4 mm thick at the pileus center, White (liii), turning slightly Sulphur Yellow (v25f) when old and injured; taste mild to slightly acrid, moderately acrid in gills; no distinct odor. Spore print Pale Cream to Cream (Romagnesi iia–iib).

Basidiospores [100/5/5] (6.6–)6.9–7.9(–8.1) × (5.8–)6.0–6.6(–7.3) µm, Q = (1.03–)1.11–1.24(–1.28) (**Q** =1.19 ± 0.05), subglobose to broadly ellipsoid, verrucose; ornamentation amyloid, composed of warts mostly fused into fine lines, cristulate to subreticulate, with some isolated verrucae, less than 1.0 µm high; plage distinctly amyloid. Basidia 26–34 × 8–13 µm, clavate, inflated towards upper half, 4–spored, projecting 5–10 µm beyond hymenium, hyaline, smooth; sterigmata 2–4 µm, pointed, straight to slightly curved. Pleurocystidia few, 43–56 × 6–10 µm, originating in subhymenium, projecting 25–30 µm beyond hymenium, thin–walled, clavate to subfusiform, with refractive contents grayish in SV in middle and lower parts; apices obtuse, often with a moniliform to papillate appendage. Cheilocystidia not observed; lamellar edge sterile. Pileipellis two layered. Epicutis a trichoderm 150–250 µm thick, composed of 3–4 µm thick, slender hyaline hyphae, occasionally with intracellular pigmentation particles. Pileocystidia numerous, 70–130 × 6–8 µm, originating in subcutis, clavate, slender, septate, with crystal contents ash black in SV; apices obtuse. Subcutis a cutis composed of recumbent, hyaline, 2.5–4 µm diam hyphae. Trama composed of connective hyphae 2–5 µm diam and hyaline, globose to subglobose, 25–40 µm diam sphaerocystsis. Stipitipellis a cutis not well developed, composed of thin–walled, septate,

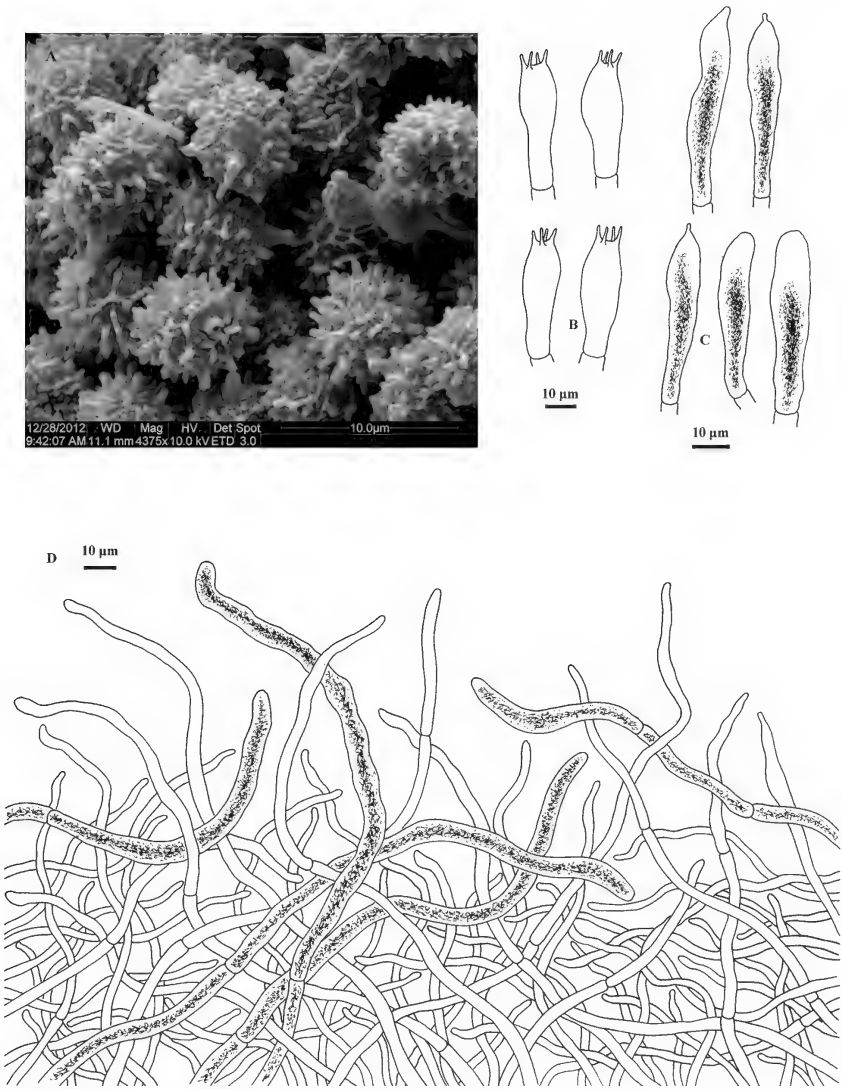

Fig. 2 *Russula changbaiensis* HMAS 262369 (holotype).
A. Basidiospores (SEM); B. Basidia; C. Pleurocystidia; D. Pileipellis.

cylindrical, hyaline hyphae 3–6 μm wide; caulocystidia absent. Clamp connections absent in all tissues.

Ecology & distribution: Solitary in conifer and conifer-broadleaf forest (often has a preference for *Abies* spp.). Distribution: China (Jilin). Season: July.

Other specimens examined: **China, Jilin Province**, Antu County, Hepingyingzi, 43°07′N 128°54′E, alt. 882 m, in mixed coniferous-broadleaf forest, 21.VII.2010, L.D. Guo, X. Sun, G.J. Li & L.J. Xie CBS20100044 (HMAS262381, GenBank KC412164); CBS20100514 (HMAS262376, GenBank KC412163); Changbai Mountains Forest Ecological System Research Center, 43°23′′N 128°05′E, alt. 811 m, in mixed coniferous-broadleaf forest, 25.VII.2010, X. Sun & G.J. Li CBS20100299(HMAS262355, GenBank KC412161).

Comments: The combination of slender basidiomata, red tinged pileus with slightly striate margin, separable pileipellis, small basidia, few short pleurocystidia, and pluriseptate dermatocystidia placed this new species in sect. *Rhodellinae* Romagn. in *Russula* subg. *Tenellula* Romagn. *Russula changbaiensis* is characterized by its slightly yellowing context, cream spore print, dermatocystidia with crystal contents, and coniferous habitat. The nine other species with a reddish tinged pileus in sect. *Rhodellinae* are *R. arpalices* Sarnari, *R. conviviales* Sarnari, *R. font-queri* Singer, *R. impolita* (Romagn.) Bon, *R. melzeri* Zvára, *R. pseudoimpolita* Sarnari, *R. puellula* Ebbesen et al., *R. rhodella* E.-J. Gilbert, and *R. zonatula* Ebbesen & Jul. Schäff. (Romagnesi 1967, 1985; Sarnari 2005). All are differentiated from *R. changbaiensis* as follows: *Russula arpalices* has a strongly yellowing context and distinctive *Pelargonium*-like odor; *R. conviviales* has an ocher spore print, partly subulate epicutis terminal cells, and wider pileocystidia (8–12 μm, Sarnari 2005) with golden yellow contents; *R. font-queri* has obviously yellowish-orange tinged pileus and lamellae, pale red tinged stipe, yellow spore print, context with a taste of old fruit, longer basidia (28–50 × 8.5–12.5 μm, Romagnesi 1967), and pleurocystidia (40–85 × 7–12 μm, Romagnesi 1967); *R. impolita* has an opaque pileus cutis, fruity odor, spores with isolated warts, and finely encrusted dermatocystidia; *R. melzeri* has an epicutis composed of large and short hyphal cells growing from a subcutis made up of sphaerocyst-like cells; *R. pseudoimpolita* has a velutinous pileus, barely changing context, ampullaceous pileipellis hyphal terminals, and association with *Quercus; R. puellula* has larger hyaline basidia, slender dermatocystidia (3–6 μm, Sarnari 2005), and an association with beech and oak deciduous forest; *R. rhodella* has an ocher to pale yellow spore print, and isolated spore ornamentation; *R. zonatula* is recognized for its obviously discolored pileus, yellowish spore print, isolated spore ornamentation, pleurocystidia over 90 μm long, and an association with beech forest (Romagnesi 1967, 1985; Sarnari 2005).

Russula taxa with reddish tinged pilei originally described from China are *R. chichuensis* W.F. Chiu, *R. griseocarnosa* X.H. Wang et al., *R. handelii* Singer, *R. jilinensis* G.J. Li & H.A. Wen, *R. minutula* var. *minor* Z.S. Bi, *R. taliensis* W.F. Chiu, *R. pseudovesca* J.Z. Ying, *R. punicea* W.F. Chiu, and *R. zhejiangensis* G.J. Li & H.A. Wen. It is easy to discriminate between these species and *R. changbaiensis: R. chichuensis* has a white spore print, large pleurocystidia ≤130

μm long, and no pileocystidia; *R. griseocarnosa* differs in the larger pileus (5–12 cm diam.), slightly pinkish stipe, and water-soaked grayish context; *R. handelii* has hyaline pleurocystidia and a stipitipellis with numerous caulocystidia; *R. jilinensis* has a yellowish green tinged pileus center, mild tasting context, and ocher to yellow spore print; *R. minutula* var. *minor* differs in the small (0.8–2.0 cm) pileus and pileipellis with primordial hyphae but without pileocystidia; *R. taliensis* is recognized by its pruinose opaque pileus, yellowish spore print, and spores with rare and isolated ornamentation; *R. pseudovesca* can be clearly perceived by its whitish spore print, numerous pleurocystidia >80 μm in length, and pileocystidia with 0.9–1.8 μm thick cell walls; *R. punicea* has a strongly acrid context that does not change color when old and injured; and

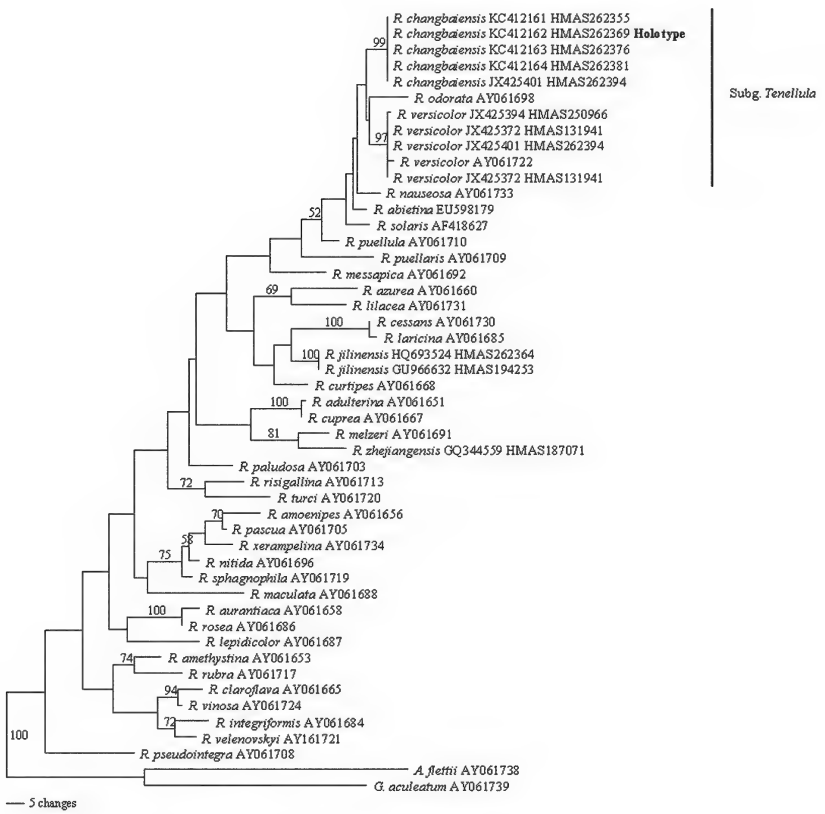

FIG. 3: Phylogenetic relationship tree of *Russula changbaiensis* and other related *Russula* species inferred from the dataset of ITS1-5.8S-ITS2 sequences. Consistency index (CI) = 0.486, retention index (RI) = 0.647, and tree length (TL) = 842. MP bootstrap support values >50% are indicated above the branches.

R. zhejiangensis has a bright red pileus with dark center, dark cream to ocher spore print, longer (≤74 μm) pleurocystidia, and a habitat of subtropical evergreen forest (Singer 1935, Chiu 1945, Bi & Li 1986, Ying 1989, Wang et al. 2009, Li et al. 2011, 2012).

Phylogenetic results

The dataset comprised 38 *Russula* taxa with particular attention paid to closely related *Russula* subg. *Tenellula* members and other red-capped *Russula* species. The sequence length was 534 nucleotides. In the MP analysis of ITS1-5.8S-ITS2 sequences, 264 characters were constant, 185 variable characters were parsimony-informative, and 85 characters were parsimony-uninformative. In the phylogenetic tree (Fig. 3), *R. changbaiensis* sequences formed a conspicuous clade, showing enough phylogenetic distance to support them as an independent taxon. *Russula changbaiensis* and most members of subg. *Tenellula* form a clade with a moderate bootstrap support of 52%, the same support as cited in Miller & Buyck (2002). The molecular analyses do support *R. changbaiensis* within subg. *Tenellula.* However, the phylogenetic relationships between *R. changbaiensis* and other subg. *Tenellula* taxa are still unclear; no stable topology can be established because there are so few corresponding sequences from dependably morphologically identified specimens.

Acknowledgments

The authors are grateful to Dr. T. Lebel (National Herbarium of Victoria, Royal Botanic Gardens Melbourne) and Dr. K. Das (Botanical Survey of India, Sikkim Himalayan Regional Centre) for carefully reviewing the manuscript; to Ms. H.M. Lü, Ms. A.R. Yan and Dr. T.Z. Wei (HMAS) for providing the herbarium specimens; to Ms. X.F. Zhu for inking in line drawings, B.A. Jaffee (University of California Davis), Dr. S.R. Pennycook (Landcare Research), Dr. L.L. Norvell (Pacific Northwest Mycology Service), and Dr. J.Y. Zhuang for improving the manuscript; to Dr. L.D. Guo, Dr. X. Sun, Dr. X.L. Wang, Mr. Z.M. Li, Ms. S.F. Yue, Mr. D. Zhao, Ms. J.X. Yang (Institute of Microbiology, CAS), Ms. L.J. Xie, Dr. C.Y. Sun and Ms. J. Zhou (National Institute of Occupational Health and Poison Control, China CDC) for assistance in collecting specimens and lab work; to Dr. C.L. Li and Ms. J.N. Liang (Institute of Microbiology, CAS) in SEM photographing. This project was supported by the National Natural Science Foundation of China (No. 30770013) and International Cooperation Program of Ministry of Science and Technology of the People's Republic of China (No. 2009DFA31160).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.279

Volume 124, pp. 279–300 *April–June 2013*

Studies on three rare coprophilous plectomycetes from Italy

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Abstract — The concept of plectomycetes is discussed and their heterogeneity emphasised. Three ascohymenial cleistothecial ascomycetes, collected or isolated from herbivore or omnivore dung in damp chamber cultures, are described. *Emericella quadrilineata* and *Lasiobolidium orbiculoides* are discussed and compared morphologically with similar taxa. A key to *Lasiobolidium* and the related *Orbicula* is provided. The importance of the second worldwide isolation of *Cleistothelebolus nipigonensis* and the difficulties of distinguishing it from *Pseudeurotium* species are stressed. The Italian collection of *C. nipigonensis* from canid dung is compared with the original strain from wolf, and its epidermoid peridial tissue is regarded as one of the main morphological differentiating features from *Pseudeurotium ovale*. The morphological characteristics of the monospecific genus *Cleistothelebolus* are discussed and compared with those of *Pseudeurotiaceae* and *Thelebolaceae*, particularly with *Pseudeurotium* and *Thelebolus*. ITS and LSU rDNA sequences of the *Cleistothelebolus* isolate support its placement in *Thelebolaceae*.

Key words — coprophily, phylogeny, *Pyronemataceae*, *Thelebolales*, *Trichocomaceae*

Introduction

Our twenty-year study on fungi growing on faecal material (Doveri 2004), both in the natural state and in damp chamber cultures, has allowed us to record from Italy several species of plectomycetes, which must be regarded as an assemblage of heterogeneous *Ascomycota* characterised by small, globose or subglobose, prototunicate asci, irregularly disposed in a centrum of cleistothecial or gymnothecial ascomata (Ulloa & Hanlin 2000, Geiser et al. 2006, Stchigel & Guarro 2007, Kirk et al. 2008). Most are pleomorphic, often with a hyphomycetous asexual state, whose features have a primary taxonomic value in separating genera and families and in establishing their phylogenetic relationships. Many are saprotrophic, growing on decaying wood or vegetables, soil, and dung, while some are parasites of human and mammal tissues (Udagawa 1987).

Plectomycetes are found in different orders and families of discomycetes, pyrenomycetes, and loculoascomycetes (Cain 1956, Benny & Kimbrough 1980). Phylogenetic studies resulting from gene sequencing combined with cladistics (Sugiyama et al. 1999, Suh & Blackwell 1999) have confirmed their great heterogeneity.

The aim of this work is to provide a detailed morphological description of *Emericella quadrilineata* and *Lasiobolidium orbiculoides*, two plectomycetous fungi recently isolated by us from dung, and to analyse their relationships with similar taxa. We also include an extensive study of the second collection worldwide of the very rare *Cleistothelebolus nipigonensis*, attempting to determine, by morphological, physiological and preliminary molecular data (ITS and LSU rDNA sequences), its connection with *Thelebolaceae* Eckblad and other families with an uncertain position in *Leotiomycetes* O.E. Erikss. & Winka.

Material & methods

Collection of *Emericella quadrilineata* and *Lasiobolidium orbiculoides*

Specimens of *E. quadrilineata* (on dried sheep dung) and *L. orbiculoides* (on dried goat dung) were collected in the Tuscan archipelago (North Tirreno sea) and placed in a non-sterilised damp chamber according to Richardson & Watling (1997) and Richardson (2001) as slightly modified by Doveri (2004). Cultured material, incubated at room temperature (20–25°C) under natural light but not exposed to direct sunlight, was examined every day under a stereomicroscope. Ascomata of both species grew fast, maturing after 7 days incubation, those of *E. quadrilineata* directly on dung, while those of *L. orbiculoides* on blotting paper at the dung base, partly obscured by the thick meshes of an aerial mycelium. Only three specimens of *E. quadrilineata* were produced, which were picked up from dung by a sterile needle, utilised for the microscopic study, preserved later in a luted slide. Specimens of *L. orbiculoides* were produced in a large quantity; however, all the attempts to isolate this fungus in axenic culture were unsuccessful. Part of the material was utilised for the microscopic study and for preservation as dried material.

Isolation of *Cleistothelebolus nipigonensis*

Cleistothelebolus nipigonensis was obtained from dried canid dung collected in northern Italy, placed in a non-sterilised damp chamber, and cultured following the same methods utilised for the other two species. Mature ascomata were hardly noticed after 20 days incubation but, owing to their smallness and staying in hiding, they may have appeared a few days earlier. Ascomata were picked up from dung by a sterile needle and crushed on PDA (Potato Dextrose Agar, Difco Lab.) amended by 2 mg L^{-1} streptomycin, for primary isolation. Plates were incubated for 14–15 days at room temperature (20–25°C) under natural light. After incubation, plates were observed at the stereomicroscope (up to 100×) and germinating groups of ascospores were selected,

picked out of the agar and transferred to PDA plates using a glass needle. Pure cultures on PDA were used to obtain single-spore isolates.

100 µl of a water suspension of ascospores were placed on WA (Water Agar, 15 g dissolved in 1 L of distilled water) plates by a sterile micropipette and incubated at 25 ± 2°C 12h/12h light/darkness. Monoascosporic isolations were transferred to PDA.

Morphological studies

Emericella quadrilineata, *L. orbiculoides* and *C. nipigonensis* were examined microscopically in mounts of water, Congo red, and methyl blue or cotton blue in lactic acid. Fifty ascospores discharged from mature asci derived from at least two ascomata were measured in water.

Cultural studies

To assess the effect of different substrates on the growth of *C. nipigonensis*, a 6 mm diameter agar disk, collected from the actively growing margin of PDA colonies, was placed in the centre of 90 mm Petri dishes containing CMA (Corn Meal Agar, Difco Lab.), MA (Malt Agar, Difco Lab.), NA (Nutrient Agar, Difco Lab.), PDA, V8 agar (Gams et al. 1998), and Emerson YpSs agar (Yeast protein Soluble starch Agar) (Gams et al. 1998). Five plates of each medium were incubated at 24 ± 2°C 12h/12h light/darkness. Radial growth of the colonies was recorded at different times from the second day of incubation. Regression and variance analysis of growth curves were performed by using SigmaPlot package (SPSS Inc., Chicago, IL) and Graphpad Prism 5.02 Software. $P \leq 0.05$ was assumed as significant level.

Molecular studies

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING — Mycelium for DNA extraction, produced on PDA plates overlaid with a cellophane membrane at 25°C, was scraped from the plates with a sterile scalpel, placed in a 1.5 ml tube (0.15 g) and extracted using the DNeasy® Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) was used to amplify the LSU and the ITS regions of the nuclear ribosomal DNA, employing the following primers: LROR and LR3 for the first 650 kb of the LSU gene and ITS1 and ITS4 for the ITS region (Gardes & Bruns 1993, White et al. 1990). Amplification reaction mixtures contained 25–50 ng of template DNA, GoTaq®Green Master Mix (Promega) 1X and 0.5 mM of each primer in a volume of 50 µL (Doveri et al. 2010). Amplification was performed in a GeneAmp® PCR System 2400 (Perkin Elmer) using the following parameters: for LSU initial denaturation step at 94°C for 5 min, 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 2 min, final extension of 72°C for 7 min; for ITS initial denaturation step at 94°C for 1 min, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 54°C for 1 min and extension at 72°C for 1 min, final extension of 72°C for 4 min. After the final extension of 72°C, reactions were held at 4°C. PCR products were purified by the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocol and submitted to sequencing. Samples to be sequenced were processed by the DNA Sequence Facility at the Bio Molecular Research (BMR), Servizio di Sequenziamento – CRIBI, University of Padova (Italy).

Results

Cultural studies

Equations resulting from regression analysis obtained for each medium were all highly significant ($R^2 \geq 0.880$, $P < 0.001$). From slopes comparison, growth rate of *Cleistothelebolus nipigonensis* on YpSs ($a = 1.7$ mm d^{-1}) was statistically higher than on all the other media ($P \leq 0.001$) (data not shown).

Molecular study

Amplifications produced sequence lengths of about 600 bp (ITS) and 650 bp (LSU). Comparison of our ITS sequence (KC492060) with those deposited in GenBank resulted in high similarity percentages (96%) with *Antarctomyces* Stchigel & Guarro and other uncultured thelebolaceous fungi. Comparison of the LSU sequence (KC492061) within the same database confirmed the close relationship between *C. nipigonensis* and two species of *Thelebolus* (*T. ellipsoideus* and *T. globosus*, 97% similarity).

Taxonomy and discussion

Emericella quadrilineata (Thom & Raper) C.R. Benj., Mycologia 47: 680, 1955 Pl. 1

Ascomata cleistothecioid, 300–350 µm diam., globose, non-ostiolate, smooth, purple-red but appearing straw coloured and slightly granulose-reticulate at first sight, as surrounded and obscured by dense yellowish masses of Hülle cells and sparse hyphae.

Hülle cells terminal, globose, exceptionally ellipsoidal, 15–25 µm diam., hyaline in the middle, with yellowish, up to 5 µm thick walls, arising out of hyaline, branched, septate, slightly encrusted hyphae, not forming a pseudostalk at the peridial base; Peridium thin-walled, purple-red, a layer of firmly pressed, narrow (about 1 µm diam.), purple-red hyphae; paraphyses absent; asci irregularly disposed, 8-spored, globose to broadly ellipsoidal, 8.5–11 × 8–9 µm, thin-walled, lacking an apical apparatus, evanescent; ascospores oblate, globose in frontal view, ellipsoidal in side view, 4–5(–5.5) × 3–3.5 µm, bivalved, hyaline at first, becoming purple-red, smooth, with four slightly raised equatorial crests, less than 0.5 µm wide, seemingly with an entire margin; asexual state only some hyaline, globose, echinulate conidia, about 3 µm diam., observed in association with ascomata.

Ecology & Distribution — Three specimens collected from sheep (*Ovis aries*) dung in a damp chamber culture. April. Rare. Known from one island coastal site in Italy, possibly cosmopolitan. Usually isolated from soil.

Specimen examined — **ITALY**, **Tuscany**, **Livorno**, Capraia island, 9°49′13″N 43°01′29″E, 50 m a.s.l., 11.IV.2011, leg. L. Levorato (Herbarium F. Doveri 007.12).

Most plectomycetes are distributed in *Eurotiomycetidae* Doweld, particularly in *Eurotiales* G.W. Martin ex Benny & Kimbr. and *Onygenales* Cif. ex Benny

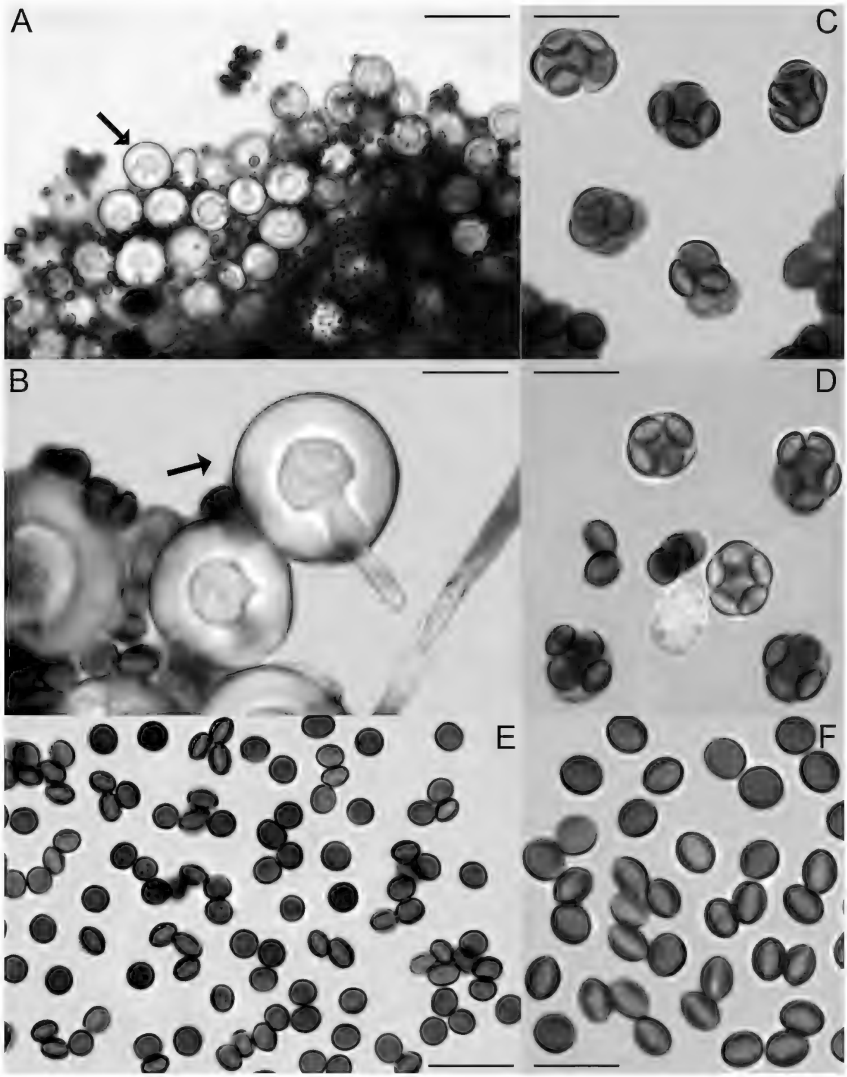


Plate 1. *Emericella quadrilineata* (Herbarium F. Doveri 007.12): A–B, clusters of Hülle cells (arrows) around the peridial wall; C, asci with conglobate ascospores; D, asci in different stages of maturity and some free ascospores; E–F, ascospores. Scale bars: A = 40 µm; B–D = 10 µm; E = 12 µm; F = 8 µm.

& Kimbr. Besides the cleistothecial ascomata and evanescent asci, these two orders share a thin, variously structured, usually brightly coloured or pale peridium, absence of an hamathecium, and small, usually brightly coloured,

one-celled, oblate ascospores often with equatorial ornamentations (Kirk et al. 2008). Blastic phialoconidiogenesis distinguishes *Eurotiales* (von Arx 1987) from *Onygenales*, where a thallic conidiogenesis produces both arthro- and aleurioconidia (Benny & Kimbrough 1980, Kirk et al. 2008).

In *Eurotiales, Trichocomaceae* E. Fisch. (= *Eurotiaceae* Clem. & Shear) encompasses several genera (including *Emericella* Berk.) characterised by brightly coloured ascomata with a cellular or hyphal peridium, often catenulate asci, usually saturnine (with equatorial crests) ascospores (Benny & Kimbrough 1980, Malloch 1985), and *Aspergillus* P. Micheli ex Link, *Paecilomyces* Bainier, or *Penicillium* Link asexual states (Malloch & Cain 1972, Kirk et al. 2008). They are cosmopolitan and usually soil inhabitants or saprotrophs on decaying plants; some are medically or industrially important as producers of antibiotics or toxins, sometimes behaving as opportunistic pathogens (Cannon & Kirk 2007) or agents of food spoilage (Geiser et al. 2006). Molecular phylogenetic studies (Berbee et al. 1995, Ogawa et al. 1997, Sugiyama 1998) have demonstrated that *Trichocomaceae* is monophyletic.

Berkeley (1857) erected *Emericella* for *E. variecolor* Berk. & Broome, a species with purplish long-spiny bordered ascospores and small cleistothecial ascomata supported by a spongy column, from which numerous threads extend, terminating in large globose cells. These stromatic (Malloch & Cain 1972), thick-walled, intercalary or end cells surrounding cleistothecia (Ulloa & Hanlin 2000, Geiser et al. 2006, Kirk et al. 2008) are called "Hülle cells" (Malloch 1985). In *Trichocomaceae* they are typical of *Fennellia* B.J. Wiley & E.G. Simmons and *Emericella*; *Fennellia* has yellow or green, often confluent ascomata, hyaline or pale ascospores, and often elongated Hülle cells (Wiley & Simmons 1973, Locquin-Linard 1990, Guarro et al. 2012), and *Emericella* has usually discrete, red or purplish to dark brown cleistothecia with a peridium of compressed hyphae, and oblate, purple-red, orange or violet, rarely pale ascospores usually ornamented with equatorial ridges. *Emericella* also has globose Hülle cells and an *Aspergillus* asexual state (von Arx 1974, Hanlin 1998, Guarro et al. 2012) of the so called "*A. nidulans* group" (Benjamin 1955, Christensen & Raper 1978, Christensen and States 1982, Pitt & Samson 2007), which is the equivalent of *Aspergillus* sect. *Nidulantes* (Gams et al. 1985). About forty species are recognised at the present (Guarro et al. 2012) in *Emericella;* they are airborne soil inhabitants (Stchigel & Guarro 1997, Samson et al. 2002), rarely growing elsewhere (Pitt and Samson 2007), thermotolerant, but not thermophilic (Zalar et al. 2008), and prefer warm climates and dry substrata (Samson & Mouchacca 1974). Some are opportunistic pathogens, uncommon agents of animal and human infections (Balajee et al. 2007, Verweij et al. 2008).

Emericella quadrilineata is characterised by rapid growth in culture, comparatively large ascomata, 8-spored asci, and smooth purple-red ascospores

with four low equatorial crests (Thom & Raper 1939). According to Balajee et al. (2007) and Verweij et al. (2008) the number of these inconspicuous crests can be detected only by electron microscopy, but we think it can be detected also by careful focusing under a light microscope.

Emericella acristata (Fennell & Raper) Y. Horie, *E. miyajii* Y. Horie, and *E. parvathecia* (Raper & Fennell) Malloch & Cain are morphologically indistinguishable from *E. quadrilineata* (Guarro et al. 2012) and possibly the same species: *E. acristata* was originally described without equatorial crests (Fennell & Raper 1955) but others have cited two (Ismail et al. 1995) or four low crests (Horie et al. 1996, Guarro et al. 2012); *E. miyajii* has four inconspicuous crests, and *E. parvathecia* has two (Raper & Fennel 1965).

The closely related *E. nidulans* (Eidam) Vuill. var. *nidulans* is also morphologically indistinguishable from *E. quadrilineata* except for having ascospores with two equatorial crests. Molecular studies that support microscopic examinations have helped separate the two species (Verweij et al. 2008, Matsuzawa et al. 2012). *Emericella quadrilineata,* usually isolated from soil (Thom & Raper 1939), has been also isolated sometimes from herbal drugs (Horie 1979), once from white wine (Stchigel et al. 1999), and now from dung. It is an opportunistic pathogen (Balajee et al. 2007), agent of onychomycosis (Gugnani et al. 2004) and rarely causes invasive aspergillosis (Drakos et al. 1993, Verweij et al. 2008). *Emericella nidulans*, which is thermotolerant (Chen and Chen 1991), cellulolytic (Reese & Downing 1951), possibly ligninolytic (Bull & Carter 1973), and opportunistically pathogenic (Balajee et al. 2007, Verweij et al. 2008) has been isolated not only from plants (Benjamin 1955), rotting wood (Eidam, 1883, Lumley et. al. 2000), and soil (Chen & Chen 1991) but also from rabbit (Benjamin 1955) and barking deer (Piasai & Manoch 2009) dung, goat droppings, pellets of free-living birds, and fresh human faeces (Domsch et al. 1993).

We know very few records of other *Emericella* species from dung, apart from *E. foveolata* Y. Horie from armadillo (Horie et al. 1996) and *E. rugulosa* (Thom & Raper) C.R. Benj. from rat (Jeamjitt et al. 2007). *Aspergillus recurvatus* Raper & Fennell, lacking a sexual state but related to *Emericella* as belonging to the *nidulans*-group (Raper & Fennell 1965), was isolated from lizard dung.

Lasiobolidium orbiculoides Malloch & Benny, Mycologia 65: 655, 1973 Pl. 2–3

Ascomata cleistothecioid, 200–500 µm diam., globose to broadly ellipsoidal, semi-membranous, yellowish at first, becoming yellow-ochraceous, finally brownish, granulose due to protruding groups of peridial cells, fully covered by dense flexuous, ≤4 mm long hairs.

Peridium two-layered, pseudoparenchymatous: endostratum with textura angularis composed of pale, thin-walled, polygonal cells, 5–8 µm diam.,

exostratum thin, weakly dextrinoid, with textura globulosa–angularis composed of thick-walled, yellowish brown cells, 12–28 × 11–22 µm, interspaced with some irregularly lobed cells; HAIRS arising from the outermost exoperidial cells, 6–7.5 µm diam., yellowish, smooth, thick-walled (up to 3 µm), wavy, sometimes slightly coiled, sparsely septate, more densely septate at their base, with rounded tips and an usually swollen, bulbous, sometimes lobed base, 10–20 µm diam; PARAPHYSES apparently scarce and short, soon vanishing, mixed with asci, 2–3 µm diam., not enlarged at the apex, septate, often branched in the lower portion, filled with a few pigments; ASCI irregularly disposed, ephemeral, unitunicate, non-amyloid, inoperculate, 8-spored, cylindrical, 80–90 × 10–11 µm, thin-walled, rounded at the apex, without an apical apparatus, with a very short, slightly lobate stalk; ASCOSPORES regularly uniseriate, oblate, 11–12.5 × 9.5–11 × (7.5)8.5–10 µm, globose to subglobose in frontal view, broadly ellipsoidal in side view, hyaline to pale yellowish, smooth, thick-walled, uninucleate, with a de Bary bubble in aqueous media, forming a powdery mass at maturity, when asci disintegrate; ASEXUAL STATE unknown.

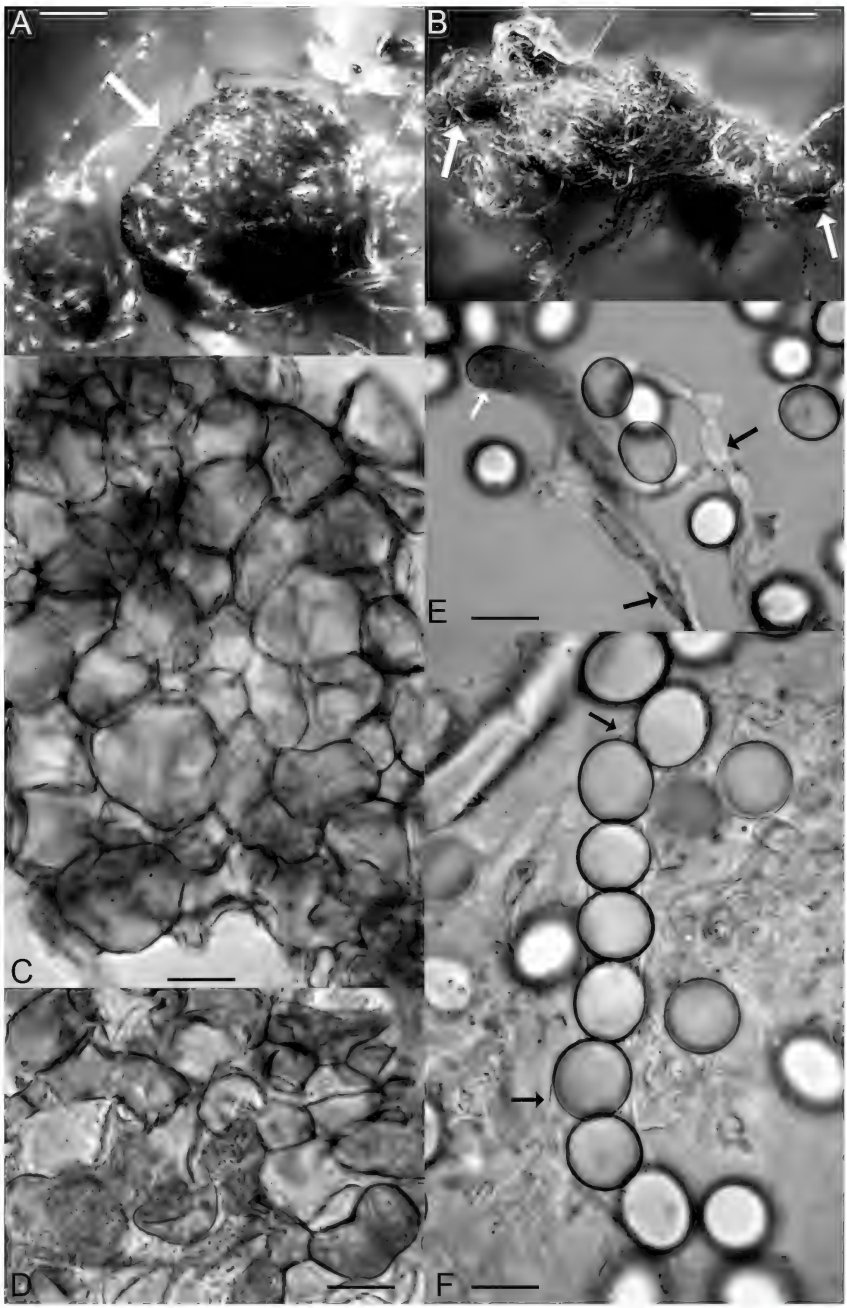
ECOLOGY & DISTRIBUTION — Hundreds of gregarious, superficial specimens collected from blotting paper at the base of goat (*Capra hircus*) dung in a damp chamber culture. April. Rare. Known from one island coastal site in Italy, possibly cosmopolitan, also known from other herbivore dung, exceptionally from soil.

SPECIMEN EXAMINED — **ITALY**, **TUSCANY**, **Livorno**, Capraia island, 9°49′13″N 43°01′29″E, 50 m a.s.l., 11.IV.2011, leg. L. Levorato (Herbarium F. Doveri 006.12).

Malloch & Cain (1971) erected the monospecific genus *Lasiobolidium*, describing *L. spirale* as a non-stromatic plectomycete with cleistothecial ascomata, a differentiated peridium with helically coiled appendages, ephemeral clavate asci, and hyaline, ellipsoidal ascospores. Malloch & Benny (1973) later described a second species, *L. orbiculoides*, so called for its strong resemblance to the monospecific genus *Orbicula* Cooke.

Lasiobolidium and *Orbicula* were initially placed (Malloch & Cain 1971, Malloch & Benny 1973, Benny & Kimbrough 1980) in *Eoterfeziaceae* G.F. Atk., now regarded as a plectomycete family with an uncertain position in *Pezizomycotina* O.E. Eriksss. & Winka (Cannon & Kirk 2007, Kirk et al. 2008, Lumbsch & Huhndorf 2010). Jeng & Krug (1976) later transferred the genera to the tribe *Theleboleae* Korf (= *Thelebolaceae*) of *Pyronemataceae* Corda (*Pezizales* J. Schröt.), but phylogenetic studies (Hansen et al. 2005, Hansen

PLATE 2. *Lasiobolidium orbiculoides* (Herbarium F. Doveri 006.12): A–B, ascomata (arrows) in culture; C–D, details of exoperidium; E, free ascospores between paraphyses (black arrows) and an immature ascus (white arrow); F, uniseriate ascospores inside a thin-walled (arrows) 8-spored ascus. Scale bars: A = 100 µm; B = 500 µm; C, E = 10 µm; D = 15 µm; F = 8 µm.


A
B
C
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E
F

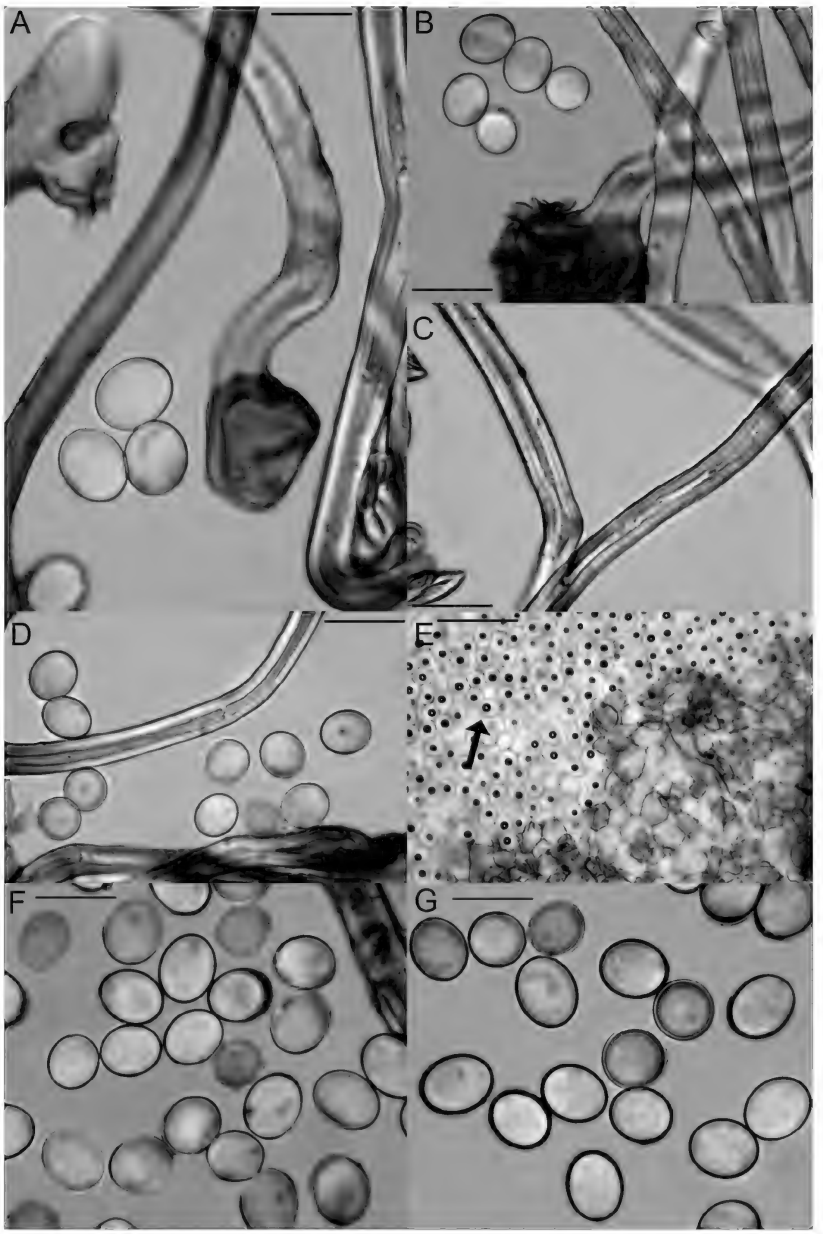


Plate 3. *Lasiobolidium orbiculoides* (Herbarium F. Doveri 006.12): A–D, details of hairs and some free ascospores; E, ascospores containing de Bary bubbles in aqueous medium; F–G, ascospores. Scale bars: A = 10 µm; B–D = 15 µm; E = 50 µm; F–G = 12 µm.

& Pfister 2006, Perry et al. 2007) place them in *Pyronemataceae*, a family of apothecioid discomycetes, with a minority of cleistothecial fungi evolved from an apothecial ancestor (Hansen et al. 2005).

Lasiobolidium orbiculoides is characterised by fast growth in culture, wavy peridial hairs, cylindrical short-stalked asci, and oblate medium-sized ascospores. The morphological characteristics of our collection fully match the protologue, and its ascospores contain a large de Bary bubble when mounted in aqueous media, a feature also reported by others (Janex-Favre & Locquin-Linard 1979, Bell 2005).

The main differences between *L. orbiculoides*, six other recognised *Lasiobolidium* species (Kirk et al. 2008), and *Orbicula parietina* (Schrad.) S. Hughes are reported in the following key, partly based on Yaguchi et al. (1996) and Guarro et al. (2012):

Worldwide key to *Lasiobolidium* and *Orbicula*

1a. Asci cylindrical. Ascospores uniseriate **2**

1b. Asci subglobose to claviform. Ascospores biseriate to conglobate, ellipsoidal **4**

2a. Ascospores broadly ellipsoidal, 11–12 × 8–9 µm (Moustafa & Ezz-Eldin 1989) ..... ***L. aegyptiacum***

2b. Ascospores oblate **3**

3a. Cleistothecia usually not flattened at their bases. Peridium thin, appearing one-layered, covered all over with hairy, wavy or sometimes irregularly coiled appendages. Asci short- or non-stalked. Ascospores 11–12.5 × 9.5–11 × 8.5–10 µm (9.8–14 × 9–12 µm; Malloch & Benny 1973). From dung, rarely from soil and vegetable material ..... ***L. orbiculoides***

3b. Cleistothecia often with a flattened base. Peridium thicker, two-layered. Hairy appendages hyphoid, present at the base only. Asci long-stalked. Ascospores 8–10.5 × 8–10.5 × 7–9 µm (7–13 × 8–10 µm; Hughes 1951). From several kinds of dung or vegetable material ..... ***O. parietina***

4a. Ascospores longer than 10 µm **5**

4b. Ascospores shorter **6**

5a. Hairs straight or somewhat flexuous, nodulose, septate, 2.5–3 µm diam. Asci subglobose or broadly clavate. Ascospores slightly warted, 18–24 × 12–18 µm (Yaguchi et al. 1996). From soil ..... ***L. gracile***

5b. Hairs helically coiled, non-septate, 5–10.5 µm diam. Asci clavate. Ascospores smooth, 12–17 × 9–12 µm (Malloch & Cain 1971). From dung, once from vegetables ..... ***L. spirale***

6a. Hairs helical, non-septate. Ascospores 8–9 × 5–6 µm (Locquin-Linard 1983). From goat and sheep dung ..... ***L. helicoideum***

6b. Hairs different in shape **7**

7a. Hairs uncinate, not septate. Ascospores 8–10 × 5.5–7 µm (Locquin-Linard 1983). From gazelle dung. ..... ***L. recurvatum***

7b. Hairs wavy, septate. Ascospores 9–10 × 5.5–7 µm (Locquin-Linard 1983). From camel and other ungulate dung ..... ***L. fallax***

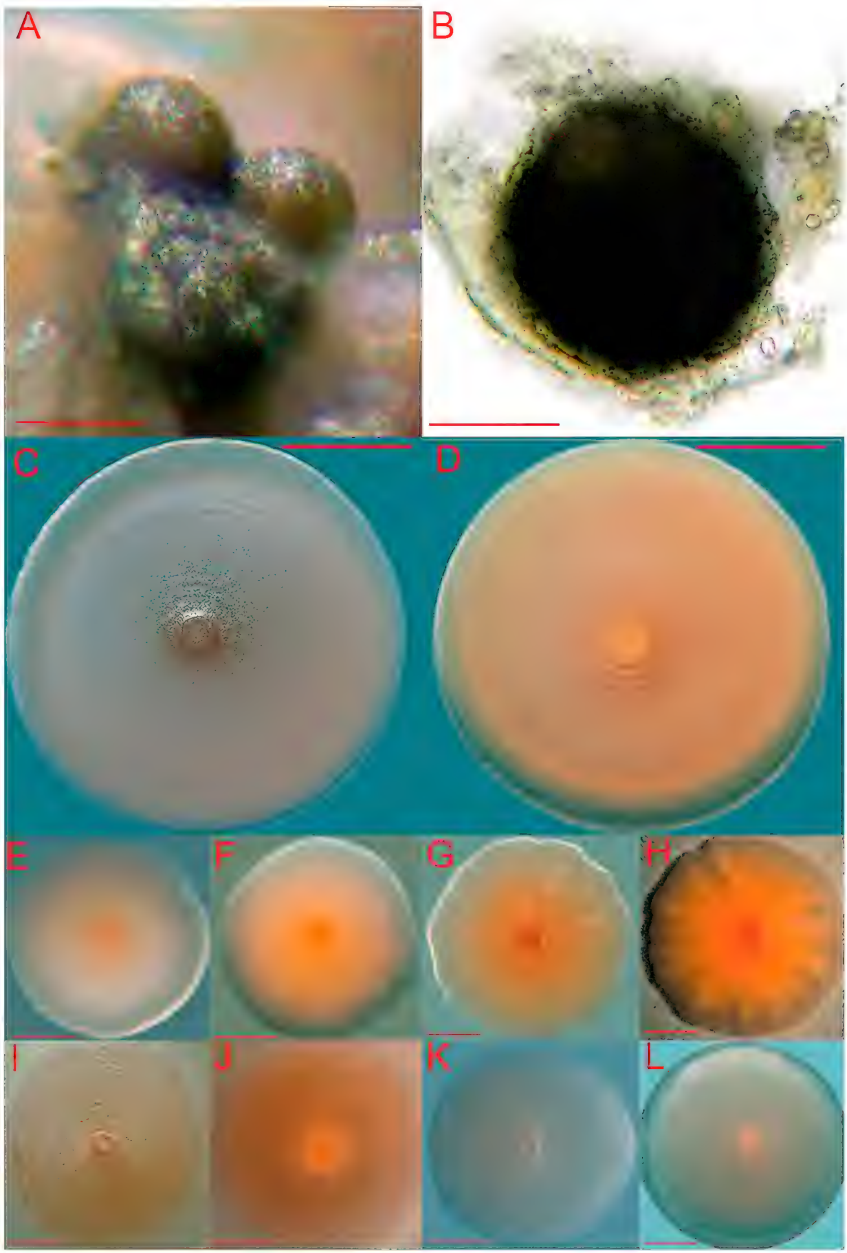
Guarro et al. (2012) regard *L. aegyptiacum* Mustafa & Ezz-Eldin is 'practically indistinguishable' from *L. orbiculoides*, but Mustafa & Ezz-Eldin (1989) originally described *L. aegyptiacum* with broadly ellipsoidal ascospores, quite different from the oblate ascospores of the latter.

Although *Lasiobolidium* is regarded as coprophilous (Kirk et al. 2008) only *L. fallax* Locq.-Lin., *L. helicoideum* Locq.-Lin., and *L. recurvatum* Locq.-Lin. have been isolated solely from dung (Locquin-Linard 1983, Abdullah & Alutby 1999). *Lasiobolidium orbiculoides* has been recorded from deer (Malloch & Benny 1973), gazelle (Janex-Favre & Locquin-Linard 1979), donkey (Abdullah & Alutby 1999), goat (Elshafie 2005), and possibly marsupial dung (Bell 2005) as well as from cultivated soil (Horie et al. 1992). Moustafa & Sharkas (1982) proved the cellulolytic activity of *L. orbiculoides* isolated from soil and grown in culture on filter paper. Its growth in our culture on blotting paper around the dung base confirms this activity. Simple or compound cellulose is found in plants and their derivatives, soil, and dung, so it is understandable that some cellulolytic fungi can develop on all these substrata without distinction, and it is possible that all other *Lasiobolidium* species are cellulolytic.

Cleistothelebolus nipigonensis Malloch & Cain, Can. J. Bot. 49: 851, 1971 Pl. 4–5

Colonies on Emerson YpSs medium attaining 60 mm diam. at 18 days, doughy-waxy, pink, with thin and soft concentric rings, which are more patent and brown shaded in the centre, margin even, reverse deeper pink, with clearly outlined central rings due to the presence of immersed, brown ascomata (YpSs medium resulted the best medium, suggesting complex nutrient requirements); Colonies on PDA and V8 juice morphologically similar to colonies on YpSs; Colonies on MA with radial soft grooves and with a wavy and sublobate margin, reverse deeper pink with more patent concentric rings and radial grooves; Colonies on NA very pale pink, with an indented margin; Ascomata cleistothecioid, globose, pale to dark brown at maturity, smooth, membranous, 60–80 µm diam.

Plate 4. *Cleistothelebolus nipigonensis* (DS 26817): A, ascomata on dung in a damp chamber; B, ascoma in water; C–L, colonies at room temperature 18 days incubation in Petri dishes (C–D, in Emerson YpSs agar; E–F, in PDA; G–H, in MA; I–J, in V8 agar; K–L, in NA; D, F, H, J, L, reverses). Scale bars: A = 40 µm; B = 30 µm; C–F, K–L = 20 mm; G–J = 15 mm.


A
B
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PERIDIUM two–layered, pseudoparenchymatous, the inner layer hyaline, the outer brown, both a textura epidermoidea of irregularly lobed, thin-walled cells, 4–10 × 3–8 µm, sometimes interspaced with spots of textura angularis; INTERASCAL TISSUE absent; ASCI not born from croziers, irregularly disposed, evanescent, unitunicate, inoperculate, non–amyloid, 8-spored, 10–14 × 9.5–10 µm, globose or broadly ellipsoidal or even broadly clavate, then short–stipitate; ASCOSPORES conglobate, 5–5.5 × 3.5–4 µm, usually broadly ellipsoidal with rounded ends, but also broadly ovoidal (Q = 1.37–1.42; Q average = 1.39), sometimes hardly inequilateral, hyaline to pale yellow, thin-walled, lacking both germ pores and de Bary bubbles; ASEXUAL STATE filamentous, lacking phialides: HYPHAE 1–3 µm diam., thin-walled, hyaline, wavy, densely septate, splitting at septa into swollen arthrospore-like cells in older colonies, branched, with long or even short branches at right angles, sometimes diverticulate, often swollen at the septa up to 5 µm diam., abundantly vacuolate, with vacuoles particularly observable in Congo red; CONIDIA 3.5–7 × 2–3 µm, 1–celled, smooth, hyaline, thin-walled, cylindrical to ellipsoidal, with a truncate pedicel, born from very small, cylindrical conidiophores along the upper hyphal side or at the apex of short branches, usually budding.

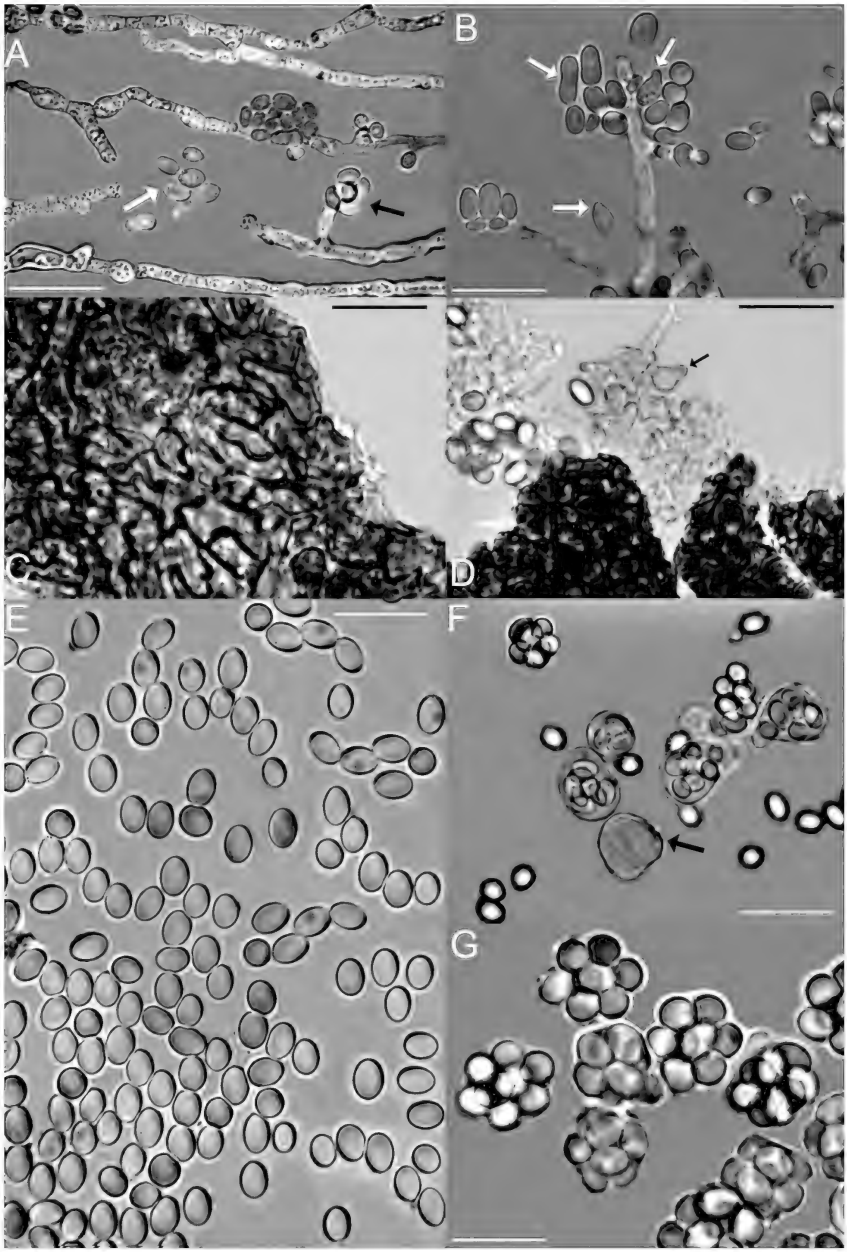
ECOLOGY & DISTRIBUTION — More than fifty, gregarious or often crowded and confluent, superficial specimens, collected from canid (possibly fox) dung in a damp chamber culture. July. Very rare, known from one mountain site (1300 m a.s.l.) in Italy, and from wolf (*Canis lupus*) dung of the type locality in Ontario (Canada).

SPECIMEN EXAMINED — **ITALY**, **VENETO**, **Vicenza**, Roana, Cesuna, 11°27′29″N 45°50′21″E, 6.VII.2008, leg. R. Cerello and G. Robich (DS 26817; GenBank KC492060, KC492061).

The name *Cleistothelebolus* means '*Thelebolus* developing cleistothecia' and emphasises the resemblance between these two coprophilous genera, which usually share ascoma and ascospore shape, size, and colour. Sharp differences, however, are noticeable, such as the presence of blastoconidia, production of true cleistothecia, irregularly disposed ephemeral asci, lack of an interascal tissue, and mesophily in *Cleistothelebolus* Malloch & Cain (Malloch & Cain 1971) versus no or a simple asexual state with production of arthrospores, cleistohymenial apothecioid ascomata, persistent asci, presence of paraphyses, and psychrophily (de Hoog et al. 2005) in *Thelebolus* Tode.

Coprotiella Jeng & J.C. Krug (*Thelebolaceae*) is similar to *Cleistothelebolus* but differs in having larger and light coloured ascomata, a peridium of a textura

PLATE 5 (right). *Cleistothelebolus nipigonensis* (DS 26817): A, free clustered conidia (white arrow) and anamorph hyphae with short conidiophores bearing conidia (black arrow); B, detail of the anamorph with some budding conidia (arrows); C, detail of exoperidial textura epidermoidea; D, exoperidial pocket of textura angularis (white arrow) beneath some free ascospores and pale



endoperidial cells (black arrow); E, ascospores; F, an empty ascus (arrow), asci with ascospores, and free ascospores; G, 8-spored asci. Scale bars: A = 25 µm; B, F = 15 µm; C, G = 10 µm; D = 20 µm; E = 12 µm.

angularis to globulosa, globose ascospores with a conspicuous de Bary bubble, and in lacking an asexual state (Jeng & Krug 1976).

We agree with Malloch & Cain (1971) that *Cleistothelebolus* has a striking morphological resemblance to *Pseudeurotium* J.F.H. Beyma, the type genus of *Pseudeurotiaceae* Malloch & Cain. This family is characterised by non-stromatic, usually dark cleistothecial ascomata with a pseudoparenchymatous peridium, (sub)globose, thin-walled, ephemeral asci, lacking apical apparatus and irregularly arranged in the centrum, absence of interascal tissue, and small usually smooth one-celled ascospores lacking germ pores. *Pseudeurotiaceae,* which have a hyphomycetous, phialidic (*Acremonium*- or *Stilbella*-like) or sympodial (*Sporothrix*- or *Teberdinia*-like) asexual state, can be isolated from wood, soil, or dung (Malloch & Cain 1970, Kirk et al. 2001, Cannon & Kirk 2007, Stchigel & Guarro 2007). *Pseudeurotium*, with its superficial, dark, globose ascomata, a peridium with a textura angularis, brown ascospores at maturity, and a sympodial asexual state, differs from *Connersia* Malloch, *Leuconeurospora* Malloch & Cain, and *Pleuroascus* Massee & E.S. Salmon. *Connersia* has hyaline, ellipsoid-reniform, inaequilateral ascospores (Booth 1961, von Arx 1987) and lacks conidia (Malloch 1974); the psychrophilic, cephalothecoid *Leuconeurospora* has ridged to reticulate ascospores (Malloch & Cain 1970, von Arx et al. 1988), and *Pleuroascus* has ascomata with helical appendages and hyaline ascospores (Massee & Salmon 1901, Malloch & Benny 1973, Barrasa & Moreno 1984). *Pseudogymnoascus* Raillo, included in *Pseudeurotiaceae* by Wang et al. (2006), is easily differentiated from *Pseudeurotium* by its gymnothecial ascomata, fusoid ascospores, and a *Geomyces* Traaen asexual state (Rice & Currah 2006).

Pseudeurotium ovale, with ovoidal-broadly ellipsoidal ascospores (Stolk 1955), differs from the majority of recognised *Pseudeurotium* species (Sogonov et al. 2005) such as *P. bakeri* C. Booth, *P. desertorum* Mouch., *P. macroglobosum* Matsush., *P. luteolum* Matsush., and *P. zonatum* J.F.H. Beyma, which have spherical ascospores (Beyma 1937; Routien 1957; Booth 1961; Mouchacca 1971; Matsushima 1975, 1996). *Pseudeurotium ovale* differs also from *P. irregulare* Lodha and *P. jaipurense* Lodha, which have irregularly ovoidal or subglobose ascospores (Lodha 1971). The ascospore shape and size in *P. ovale* are similar to those of *Cleistothelebolus nipigonensis,* which, however, can be distinguished by a "lighter colored peridium, less complex ascogenous system, and lack of phialides" (Malloch & Cain 1971). Based on our collection *C. nipigonensis* and numerous reports on *P. ovale* (Stolk 1955, Booth 1961, Udagawa 1965, Ahmad & Sultana 1973, Dennis 1981, Eriksson 1992, de Hoog et al. 2000), we will note some additional differences between these two taxa. *Cleistothelebolus* ascomata are smaller (15–80 versus 90–250 µm diam.), asci are larger (9–14 × 8–10.5 versus 7–9 × 6.5–8 µm) and often short-stipitate (rather than globose to broadly

ovoid or ellipsoid), and the peridium is two- instead of one-layered. The nature of the *Cleistothelebolus* peridium is particularly noticeable: in our collection it is formed of a TEXTURA EPIDERMOIDEA, not unmentioned but clearly depicted in the protologue (Malloch and Cain 1971). A pseudoparenchymatous peridium was also described in *P. ovale* but depicted as a TEXTURA ANGULARIS (Stolk 1955, Udagawa 1965, de Hoog et al. 2000). The habitat of the two species also differs: originally isolated from nematode cysts (Stolk 1955), *P. ovale* has been isolated from soil (Udagawa 1965), grass, wood chips, and decaying algae (Richardson 2004), but only rarely from dung (Ahmad & Sultana 1973, Eriksson 1992), whereas the only two *C. nipigonensis* collections come from wolf (the holotype) and fox (ours) dung.

Based on morphological (Kimbrough & Korf 1967, Eckblad 1968) and ultrastructural studies (Brummelen 1998), the *Thelebolaceae* were accommodated in *Pezizales*, but the placement was later questioned by rDNA sequence data (Momol & Kimbrough 1994, Momol et al. 1996). *Thelebolus* and related genera were excluded from *Pezizales* based on morphological and ultrastructural (Samuelson & Kimbrough 1978, Kimbrough 1981) and molecular phylogenetic studies (Landvik et al. 1998, Mori et al. 2000, Gernandt et al. 2001, Hansen et al. 2005). Molecular analysis of the nuclear SSU rDNA gene (de Hoog et al. 2005) confirmed the placement of *Thelebolus*, *Caccobius* Kimbr., and *Ascozonus* (Renny) E.C. Hansen within the separate family *Thelebolaceae* (*Thelebolales* P.F. Cannon), near the leotialean fungi and far from those orders to which they were assigned previously. *Thelebolaceae* now are considered allied with the *Leotiomycetes*, as stated in a recent study by Hansen & Pfister (2006) on the molecular systematics of *Pezizomycetes* O.E. Erikss. & Winka.

Cleistothelebolus was originally placed with *Lasiobolidium*, *Orbicula,* and other cleistothecial fungi, in *Eoterfeziaceae* (Malloch & Cain 1971, Benny & Kimbrough 1980), a family with an uncertain position in *Ascomycota* morphologically similar to *Pseudeurotiaceae* but with an unknown asexual state (Kirk et al. 2001, Cannon & Kirk 2007).

Cleistothelebolus was later transferred (Jeng & Krug 1976) to *Pyronemataceae* trib. *Theleboleae*, which on the whole is the equivalent of the current *Thelebolaceae*.

Landvik et al. (1997) suggested that genera other than *Thelebolus* belonging to small pezizalean families with uncertain affinities (like *Cleistothelebolus*) might be related to *Leotiales* and noted that further phylogenetic analyses are needed to explain the relationships of *Cleistothelebolus*, which Malloch & Cain (1971) originally regarded as probably evolved from *Thelebolaceae*. Lumbsch & Huhndorf (2007, 2010) suggested placing *Cleistothelebolus* within *Pyronemataceae,* but further phylogenetic studies are needed there as well. Our ITS and LSU sequence analyses support *Cleistothelebolus nipigonensis* in *Thelebolaceae*.

Acknowledgements

The authors wish to thank Prof. J. Guarro, Prof. Kevin Hyde, and the MYCOTAXON editors for critical revision of the manuscript, Gianni Robich and Lucia Levorato for providing part of the material studied, and Maurizio Forti for technical support.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.301

Volume 124, pp. 301–308 *April–June 2013*

Morphological and genetic characterisation of *Beauveria sinensis* sp. nov. from China

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Abstract —*Beauveria sinensis* sp. nov. was isolated from a larva of *Geometridae* (*Lepidoptera*) collected from Tiantangzhai, Anhui province, China. It is characterized by elongated ellipsoidal to cylindrical conidia, solitary conidiogenous cells that are cylindrical or with slightly swollen bases, white to pale pink colony in culture, and small mycelial pellets consisting of curved hyphae. Phylogenetic analyses of translation elongation factor-1 alpha (TEF), RNA polymerase II largest subunit (RPB1), and RNA polymerase II second largest subunit (RPB2) sequence data support it as a unique species and sister to *B. sungii*. It does not, however, form a monophyletic group with other elongated ellipsoidal to cylindrical conidia-producing species in *Beauveria*. Type isolates and holotype are deposited in the Research Center for Entomogenous Fungi of Anhui Agricultural University (RCEF).

Key words —*Ascomycetes*, taxonomy, entomopathogenic fungi

Introduction

Beauveria Vuill. is a globally distributed genus of soil-borne and entomopathogenic hyphomycetes. It is of particular interest as a model system for the study of entomopathogenesis and the biocontrol of pest insects (Rehner & Buckley 2005). *Beauveria* species are characterized by whorled conidiophores and dense clusters of sympodial and globose or flask-shaped short conidiogenous cells with apical denticulate rachi that give rise to one-celled hyaline conidia. Conidial shape in *Beauveria* can be globose, ellipsoidal, cylindrical, or comma-shaped.

Recent phylogenetic analyses of *Beauveria* inferred from partial sequences of RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2), translation elongation factor-1 alpha (TEF) genes, and the Bloc nuclear intergenic region (Bloc) resolved 12 clades, each of which

includes one recognized species (Rehner et al. 2011). Since then Zhang et al. (2013) have described *B. lii* Sheng L. Zhang & B. Huang from China, a species with large cylindrical to ellipsoidal conidia. In *Beauveria*, there are eight species with ellipsoidal, cylindrical, or comma-shaped conidia: *B. amorpha* (Höhn.) Minnis et al., *B. asiatica* S.A. Rehner & Humber, *B. brongniartii* (Sacc.) Petch, *B. caledonica* Bissett & Widden, *B. lii*, *B. malawiensis* S.A. Rehner & Aquino de Muro, *B. sungii* S.A. Rehner & Humber, and *B. vermiconia* de Hoog & V. Rao (Bissett & Widden 1988, de Hoog & Rao 1975, Rehner et al. 2006, 2011; Samson & Evans 1982, Shimazu & Hashimota 1988, Zhang et al. (2013). Five species with globose to subglobose conidia include the type of the genus, *B. bassiana* (Bals.-Criv.) Vuill., and *B. australis* S.A. Rehner & Humber, *B. kipukae* S.A. Rehner & Humber, *B. pseudobassiana* S.A. Rehner & Humber, and *B. varroae* S.A. Rehner & Humber (Rehner et al. 2011).

Recently, we isolated a *Beauveria* with elongated ellipsoidal to cylindrical conidia from Anhui province, China, infecting *Geometridae* larvae. The isolate possessed unique cultural and morphological characteristics and did not form a monophyletic group with other *Beauveria* species that produce cylindrical conidia. We concluded that it represents a new species and describe it here as *B. sinensis*.

Materials & methods

Collection of specimens and isolation

An entomogenous specimen (TTZ070716-26) was collected from Tiantang zhai, Jinzhai county, Anhui province, China, in July 2007. The host was identified as belonging to *Geometridae* (*Lepidoptera*). Strain RCEF3903 was isolated from this collected specimen on SDAY (1% w/v peptone, 4% w/v dextrose, 0.2% w/v yeast, 1.5% w/v agar) medium.

Strain identification

Morphological characteristics are based on cultures incubated on quarter-strength SDAY (SDAY/4) at 25 °C for 14 d. The isolated fungus was examined using classical mycological techniques based on growth rate as well as macroscopic and microscopic characteristics. The ex-type culture and a dried-culture holotype specimen has been deposited in the Research Center for Entomogenous Fungi, Anhui Agriculture University, Hefei, Anhui, China (RCEF).

DNA extraction, PCR and sequencing

Conidia were inoculated onto SDAY medium overlaid with a disc of sterilized cellophane. After incubating at 25 °C for approximately 7 days, genomic DNA was extracted from the mycelia scraped from the cellophane using modified CTAB method (Gardes & Bruns 1993, Spatafora et al. 1998). The extracted DNA was stored in sterile distilled water to a final concentration of 1–2 ng μl^{-1} and stored at –20 °C.

To perform phylogenetic analysis of the isolated *Beauveria* strains (Zhang et al. 2013; Rehner et al. 2011), the partial sequences of three nuclear loci, including TEF, RPB1,

and RPB2, were amplified as described by Rehner & Buckley (2005) and Rehner et al. (2011). After purifying PCR product using EasyPure quick gel extraction kit (TransGen Biotech), DNA sequencing was performed at Sangon Company (Shanghai, China) and the resulting sequences of RCEF3903 were submitted to GenBank.

Sequence alignment and phylogenetic analyses

DNA sequences generated in this study were assembled and edited using Codoncode Aligner (version 3.6.1). Sequences of TEF, RPB1, and RPB2 from 69 taxa (68 *Beauveria* isolates and one *Isaria tenuipes* strain as outgroup) based on the results of Zhang et al. (2013), Rehner & Buckley (2005), and Rehner et al. (2006) were downloaded form GenBank. Multiple sequence alignments for TEF, RPB1, and RPB2 constructed using Clustal X 1.81 (Thompson et al. 1997) were concatenated into a single file using the program Mafft (Katoh et al. 2002), and MEGA was used to output a Nexus file for phylogenetic analysis. The datasets were analysed using both maximum parsimony (MP) and Bayesian algorithms.

PAUP* 4.0b10 (Swofford 2002) was used to perform maximum parsimony analyses on the combined dataset of TEF, RPB1, and RPB2 with 1000 replicates of heuristic search of random sequence additions, branch swapping algorithm by tree bisection-reconnection (TBR) and MulTrees on. Insertions and deletions were minimized by direct examination and treated as missing data. Ambiguously aligned sequence regions were excluded from the data matrix before analysis. Branch support was estimated by bootstrapping using 1000 replicates with full heuristic search (Felsenstein 1985). Clades with bootstrap values ≥70% were considered strongly supported by the data.

Bayesian analyses were conducted with an online version of MrBayes 3.0b4 (Huelsenbeck et al. 2001) through the CIPRIS web portal. 1,000,000 Markov Chain Monte Carlo (MCMC) generations were performed with four chains (three cold, one heated) and trees were saved every 100 generations for a total of 100,000 trees. Analyses were repeated two times and converged close to the same value (the standard deviation of split frequencies about 0.005) and first 25% of trees were discarded as burn-in. The consensus tree with the support values for each branch constituting their posterior probability (≥95% were considered as significantly supported by the data) was combined into a single tree file. Posterior probabilities for branches receiving ≥95% support are reported below the respective branches on the single best tree from the Bayesian analysis (Fig. 1). The bootstrap values for branches supported in ≥70% are listed above branches in Fig. 1.

Results

For RCEF3903, PCR amplification yielded TEF, RPB1, and RPB2 amplicons of 1714 bp, 2866 bp, and 2118 bp. The combined alignment of the above genes included a total of 6698 characters. After ambiguously aligned positions excluded, the final alignment comprised 6028 bp (TEF: 1004; RPB1: 2860; RPB2: 2164), of which 771 were parsimony informative. Maximum parsimony analysis of 70 taxa dataset yielded 48 equally most-parsimonious trees of 2018 tree length with consistency indices (CI) of 0.7096 and retention indices (RI) of 0.9091. One of nine equally most-parsimonious trees is shown in Fig. 1 with

bootstrap values ≥70% above the relevant branches. Bayesian analysis resulted in the same topology as the MP analysis shown in Fig. 1.

The phylogenetic analysis of combined data set well supported RCEF3903 as a sister group to the *B. sungii* clade containing seven individuals. All isolates from this clade produced ellipsoidal or oblong conidia in culture (Rehner et al. 2011), and RCEF3903 differs from these seven isolates in conidial size. The molecular data and analyses also suggest a wide relationship of RCEF3903 with *B. caledonica* group, *B. amorpha* group, *B. malawiensis* group and *B. lii*.

Taxonomy

Beauveria sinensis Ming J. Chen, Z.Z. Li & B. Huang, **sp. nov.** Plates 2–7

MycoBank MB 801325

Differs from all other *Beauveria* species by its pale pink colony and production of mycelial pellets consisting of curved hyphae.

Type: China, Anhui Province, Jinzhai County, isolated from a larva of *Geometridae*, July 2007, coll. Mingjun Chen (**Holotype**, RCEF3903-DAC1; ex-type culture RCEF3903; GenBank HQ270151, JX524283, JX524284).

Etymology: *sinensis*, for the country of origin, China.

Colony on SDAY/4 attaining a diameter of 23–31 mm after 14 days at 25 °C, aerial mycelium white, dense and cottony at first, slowly becoming pale pink and producing considerable amounts of mycelial pellets on older portions, regular in the margin, reverse light yellow-brown, odor indistinct. The mycelial pellets attaining 95.0–150.0 µm in diameter after 14 days at 25 °C, and consisting of curved hyphae. Typical hyphae hyaline, smooth-walled, septate, branched, 1.8–2.5 µm diam., curved mycelium a little slighter. Conidiophores arising on aerial hyphae, similar to aerial hyphae, mostly simple. Conidiogenous cells mostly borne on conidiophores, occasionally on aerial mycelium, solitary, flask-shaped to cylindrical, 8.5–20.0 × 1.6–3.0 µm, tapering into a long slender denticulate rachis, geniculate or irregularly bent, 5.0–10.0 × 0.5–0.6 µm. Conidia elongate ellipsoidal to cylindrical, one-celled, hyaline, smooth, 3.0–5.0 × 1.5–2.0 µm (L^m = 4.1 µm, W^m = 1.7 µm, Q^m = 2.4), chlamydospores absent. Teleomorph not observed.

Discussion

We tried to amplify the Bloc loci of *B. sinensis* several times with Bloc primers described by Rehner et al. (2011), but failed to get its PCR production. The phylogenetic relationship among the *Beauveria* inferred from three nuclear loci was mainly consistent with those inferred from four nuclear loci (Rehner et al. 2011). Furthermore, Rehner et al. (2011) emphasized that each of the four loci used to reconstruct the phylogeny of *Beauveria* was effective for accurate diagnosis of all 12 species based on multiple species-specific phylogenetically

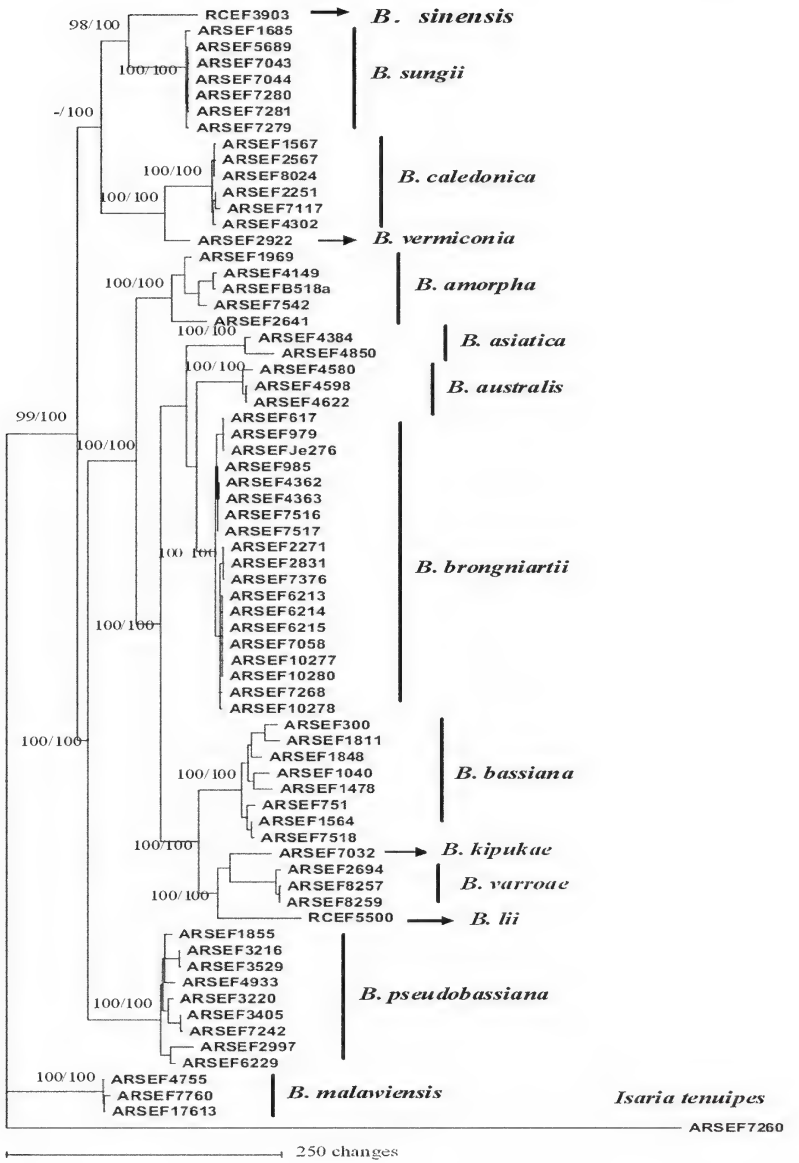
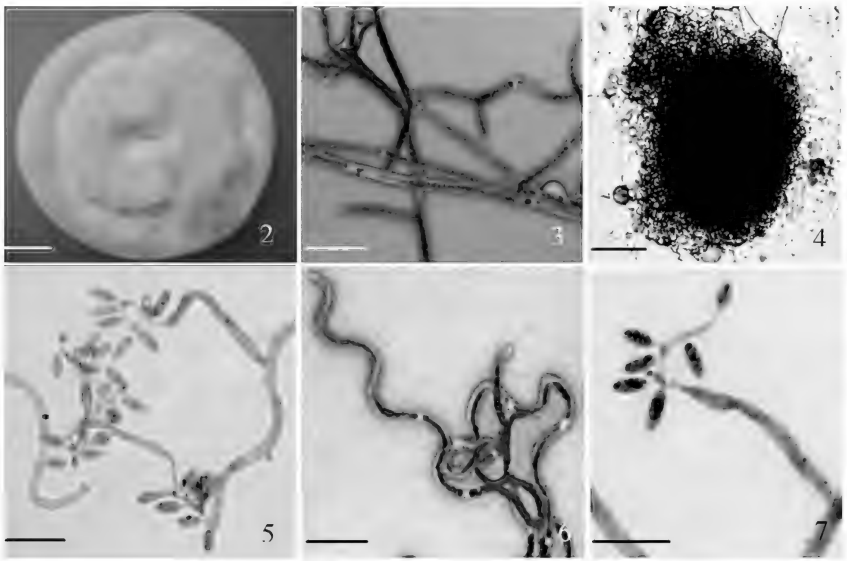


FIG. 1. A single tree from the maximum parsimony analysis showing phylogenetic relationships among species of *Beauveria* inferred from a combined dataset of TEF, RPB1, and RPB2 data. Bootstrap values ≥70% and posterior probabilities ≥95% are labeled above and below the appropriate branches.



FIGS 2–7. *Beauveria sinensis*. 2. Colony on SDAY/4, showing mycelial pellets. 3. Typical hyphae. 4. Mycelial pellets. 5. Conidiophores, conidiogenous cells, and conidia. 6. Curved hyphae. 7. Phialide and conidia. Scale bars: 2 = 50 mm; 3, 5, 6, 7 = 10 µm; 4 = 30 µm.

informative nucleotide characters. Therefore, the position of *B. sinensis* in the tree from the combined analysis of RPB1, RPB2, and TEF data is reasonable and reliable.

In culture, *B. sinensis* is distinguishable by a pale pink colony and production of mycelial pellets consisting of curved hyphae. Most species of *Beauveria* are white to buff on solid media except *B. malawiensis*, whose colonies become light pink in older portions. Mycelial pellets consisting of curved hyphae are formed by *B. sinensis* isolates but are unknown in any other *Beauveria* species. Subcultured for five generations, the isolates still produced significant amounts of mycelial pellets. The unique stable growth behavior may prove to be of taxonomic value in *Beauveria*, with additional isolates discovered.

In *Beauveria*, there were three species with ellipsoidal conidia (*B. brongniartii*, *B. asiatica*, and *B. sungii*) and four species with elongate ellipsoidal to cylindrical conidia similar to the new species (*B. amorpha*, *B. caledonica*, *B. malawiensis*, and *B. lii*). TABLE 1 provides a comparative summary of the main characters of *B. sinensis* and the other four species with elongate ellipsoidal to cylindrical conidia. Microscopically, *B. sinensis* is distinguished by conidiogenous cell shape from *B. amorpha*, *B. caledonica*, and *B. malawiensis*, all of which produce whorls of conidiogenous cells with globose to subglobose bases. *Beauveria malawiensis* is further distinguished by its short and thick rachis, *B. amorpha*

Table 1. Morphological comparisons of *Beauveria* spp. with elongated ellipsoidal to cylindrical conidia.

Species	Colony	Conidia (μm)	Conidial shape	Conidiogenous cell
B. amorpha	White to yellow	3.5–5 × 1.5–2	Cylindrical, often flattened on one side or slightly curved	Whorled, globose to subglobose base, basal part 2–4 μm
B. caledonica	White to cream to dull buff	3–5 × 1–1.8	Ellipsoidal to cylindrical	Simple to whorled, swollen base, 2.6–4.2 × 1.6–3.3 μm
B. lii	White to cream	3.1–10.1 × 1.4–3.6	Ellipsoidal to cylindrical, occasionally obovoid	Solitary, occasionally tight clusters of 2–3, base ellipsoidal to cylindrical 4.8–9.0 μm
B. malawiensis	White to pale pink, slightly zonate	3.7–4.5 × 1.3–1.9	Cylindrical	Whorled, base globose to obpyriform, 2.0–6.6 μm
B. sinensis	White to pale pink, curved mycelium in mycelial pellet	3–5 × 1.5–2	Elongate ellipsoidal to cylindrical	Solitary, slightly or no swollen base, 8.2–20 × 1.6–3.0 μm

has a characteristically shaped conidium that is often slightly curved or flattened on one side (Samson & Evans 1982), and *B. lii* produces the largest conidia (Zhang et al. 2013).

In the Bayesian analysis and maximum parsimony of TEF, RPB1, and RPB2 sequence data, *Beauveria sinensis* was the sister species to *B. sungii* group according to Rehner et al. (2011), and was distinct from other *Beauveria* species with cylindrical conidia. Thus, the phylogenetic studies also supported the recognition of *Beauveria sinensis* as a distinct species.

Acknowledgments

We are grateful to Prof. Z.Q. Liang and Dr. L. Wang for reviewing the manuscript. We also thank Drs. Shengli Zhang and Yue Cai for their kind help with molecular work. This work was supported by the Natural Science Foundation of China (No.31200114 and 31070009).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.309

Volume 124, pp. 309–321 *April–June 2013*

The lichen genus *Fissurina* (*Graphidaceae*) in Vietnam

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Abstract — Nine species of *Fissurina* from Vietnam are briefly commented on. *Fissurina dumastii, F. dumastioides, F. instabilis, F. rubiginosa,* and *F. undulata* are recorded as new for the Vietnam lichen biota. Characteristic morpho-anatomical and chemical features are described and summarized in an artificial key to all known taxa of *Fissurina* from Vietnam.

Key words — Chu Yang Sin National Park, corticolous, graphidoid, taxonomy

Introduction

Fissurina Fée (*Ascomycota*: *Ostropales*) accommodates species with mostly slit-like lirellae (Staiger 2002; Archer 2009). The diagnostic characters of the genus includes a pale to yellow-brown to olive green (rarely whitish), mostly smooth and glossy thallus, fissurine, simple to branched, immersed to prominent lirellae, uncarbonized or rarely carbonized proper exciple, clear hyaline non-amyloid hymenium, 1–8-spored asci, and hyaline, oval or narrowly to broadly ellipsoid, trans-septate to muriform, amyloid to non-amyloid, thick-walled, mostly halonate ascospores (Archer 2009; Sharma et al. 2012). *Fissurina* occurs in the tropics and presently comprises more than 100 species worldwide. Many species have recently been added to the genus as a consequence of a molecular and phylogenetic revision of the *Graphidaceae* (Rivas Plata et al. 2012; Sharma et al. 2012).

Aptroot & Sparrius (2006) recorded four *Fissurina* species — *F. marginata* (= *F. elaiocarpa*), *F. egena*, *F. incrustans*, and *F. radiata* —from different Botanical Gardens and National Parks of Vietnam. During a recent field excursion to Chu Yang Sin National Park (Vietnam), several interesting graphidoid taxa were collected, including five new records of *Fissurina* for Vietnam: *F. dumastii*, *F. dumastioides*, *F. instabilis*, *F. rubiginosa*, and *F. undulata*. Among the *Fissurina*

species in the country, *F. dumastioides* and *F. rubiginosa* produce stictic acid, whereas the others lack secondary metabolites.

This report is a preliminary attempt to assess the taxonomy of fissurine lichen species known so far from Vietnam. The synopsis clarifies the current status of the genus within the country and confirms the high diversity of graphidoid taxa in tropical rain forests. Diagnostic characters are studied from the available material. Unfortunately, many of the samples were either immature or collected in low frequency and many others that could increase the diversity considerably were sterile. In the following account nine reports of *Fissurina* are briefly described and summarized in an artificial key.

Materials & methods

Material deposited in the herbarium of the Korean Lichen Research Institute (KoLRI) was examined morphologically, anatomically, and chemically. The morphology of thallus and the ascomata were observed using a SMZ-168 dissecting microscope. Thin hand-cut sections mounted in tap water, in lactophenol cotton blue, in 5% KOH, or in Lugol's solution were observed under a Olympus BX50 compound microscope. Chemical spot tests and TLC (using solvent system C & A) were performed according to Orange et al. (2010).

Taxonomic descriptions

Fissurina dumastii Fée, Essai Crypt. Écorc.: xc, 1825. Pl. 1

Thallus corticolous, epiperidermal, corticate, to ca. 250 µm thick, glossy, smooth, continuous to uneven, greenish grey, pale green or olivaceous to dull green; cortex 15–30 µm thick; algal layer well developed, continuous, 20–35 µm thick; medulla indistinct, mostly endophloeodal, studded with crystals; calcium oxalate crystals small to moderately large, clustered or scattered; prothallus distinct, white. Vegetative propagules not seen.

Ascocarps numerous, lirelliform, ± conspicuous, immersed to erumpent, indicated by a thin line between two lips, sometimes slightly open, slightly raised and paler than the thallus, straight, curved or sinuous, often branched, 0.6–2 × 0.2–0.25 mm, *dumastii*-type (Staiger 2002); disc slit-like, narrow to sometimes open in mature apothecia, flesh-colored. Thalline margin concolorous with the thallus or slightly paler, thin to thick, ± incurved or straight, 10–40 µm thick. Proper exciple brownish to dark brown, ca. 15–50 µm thick. Epihymenium hyaline, indistinct, with greyish or brownish granules, ≤10 µm high. Hymenium hyaline, clear, to ca. 50–95 µm high, I–. Paraphyses straight to bent, ± interwoven, unbranched, with slightly thickened tips, moderately to distinctly conglutinated, 1–2 µm thick. Subhymenium indistinct to hyaline, ca. 20 µm high. Asci clavate, 8-spored, ca. 65–80 µm, I–. Ascospores uniseriate, hyaline, becoming mottled or brown in late maturity, transversely

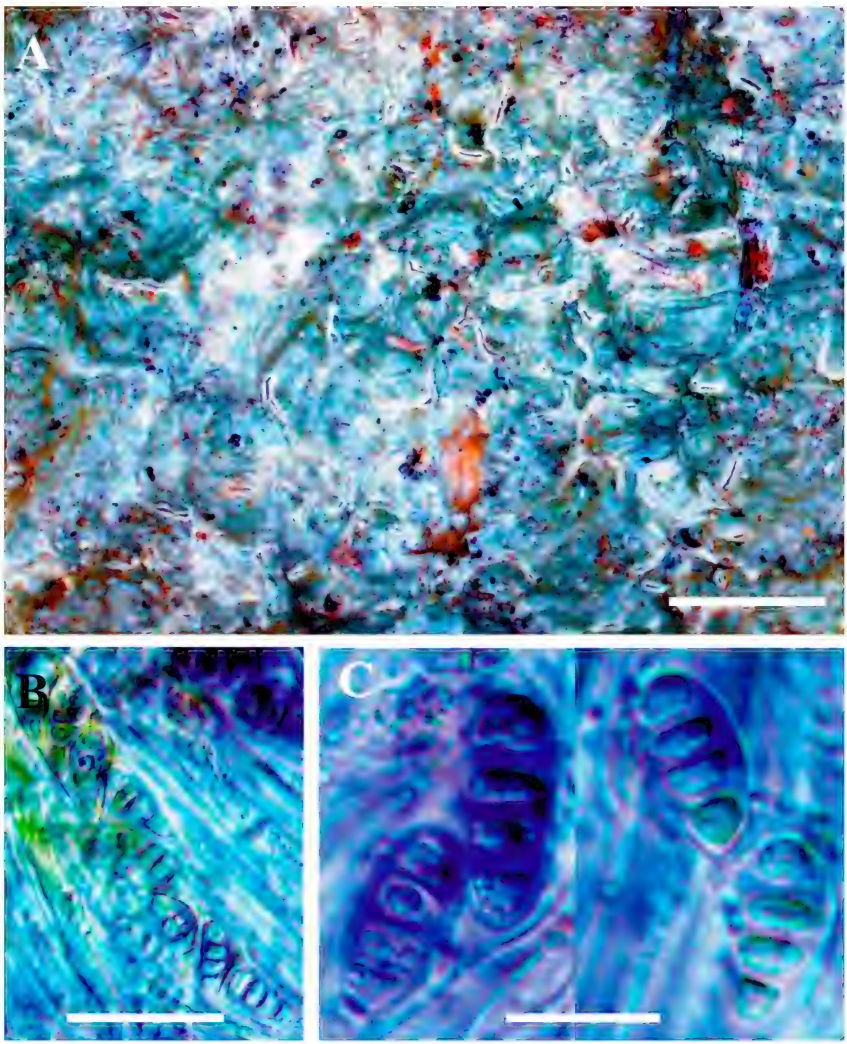

Plate 1. *Fissurina dumastii*. A. Habit. B. Ascus with ascospores. C. Ascospores. Scale bars: A = 2 mm; B = 25 µm; C = 10 µm.

septate, oblong to ellipsoidal, with ± rounded to narrowly rounded to subacute ends, thick walled, halonate, 15–18 × 4–9 µm, with 4 lenticular to ± round locules, I+ blue violet.

Chemistry: K–, PD–, C–; no lichen compound detected in TLC.

Distribution & ecology: This species has a pantropical distribution and was previously reported from Queensland, New South Wales, Indonesia,

Solomon Islands, and Brazil (Staiger 2002; Archer 2006, 2009). In Vietnam it was collected from tree trunks of an evergreen forest in the Chu Yang Sin National Park at an altitude of ca. 600 m.

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′04.4″N 108°20′39.0″E, alt. ca. 622 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen 120282 (KoLRI).

Remarks: *Fissurina dumastii* seems close to *F. insidiosa* C. Knight & Mitt. in having trans-septate ascospores and thallus lacking chemical substances, but differs in thallus and apothecial morphology. *Fissurina insidiosa* has distinct verrucose-bullate thallus and labiate lirellae. Moreover, the ascospores of *F. dumastii* show distinct amyloidity, absent in *F. insidiosa*. Another species belonging to the *dumastii* species complex is *F. analphabetica* Common & Lücking, but it has very small delicate lirellae (0.3–0.5 mm) and comparatively smaller ascospores of 15 × 7 µm (Lücking et al. 2011). *Fissurina egena* also has roof-like apothecial margins but differs in its muriform ascospores.

Fissurina dumastioides (Fink) Staiger, Biblioth. Lichenol. 85: 161, 2002. Pl. 2

Thallus corticolous, epiperidermal, corticate, to ca. 250 µm thick, glossy, smooth, continuous to uneven, green to olive-green; cortex 15–25 µm thick; algal layer well developed, continuous, 20–30 µm thick; medulla indistinct, mostly endophloeodal, studded with crystals; calcium oxalate crystals small to moderately large, clustered or scattered; prothallus distinct, white. Vegetative propagules not seen.

Ascocarps numerous, lirelliform, ± conspicuous, immersed to erumpent, indicated by a thin line between two lips, sometimes broadly open, slightly raised and paler than the thallus, straight, curved or sinuous, often branched, ≤5 mm long and 0.2–0.3 mm wide with recurved margins, *dumastii*-type (Staiger 2002); disc narrow, open in mature apothecia, greyish to flesh-colored. Thalline margin concolorous with the thallus or slightly paler, thin to thick, ± incurved or straight, 10–40 µm thick. Proper exciple brownish to dark brown, ca. 15–50 µm thick. Epihymenium hyaline, indistinct, with greyish or brownish granules, ≤10 µm high. Hymenium hyaline, clear, to ca. 50–90 µm high, I–. Paraphyses straight to bent, ± interwoven, unbranched, with slightly thickened tips, moderately to distinctly conglutinated, 1–2 µm thick. Subhymenium indistinct to hyaline, ca. 20 µm high. Asci clavate, 8-spored, ca. 60–80 µm, I–. Ascospores uniseriate, hyaline, becoming mottled or brown in late maturity, transversely septate, oblong to ellipsoidal, with ± rounded to narrowly rounded to subacute ends, thick walled, halonate, 15–20 × 4–9 µm, with 4 lenticular to ± round locules, I+ blue violet.

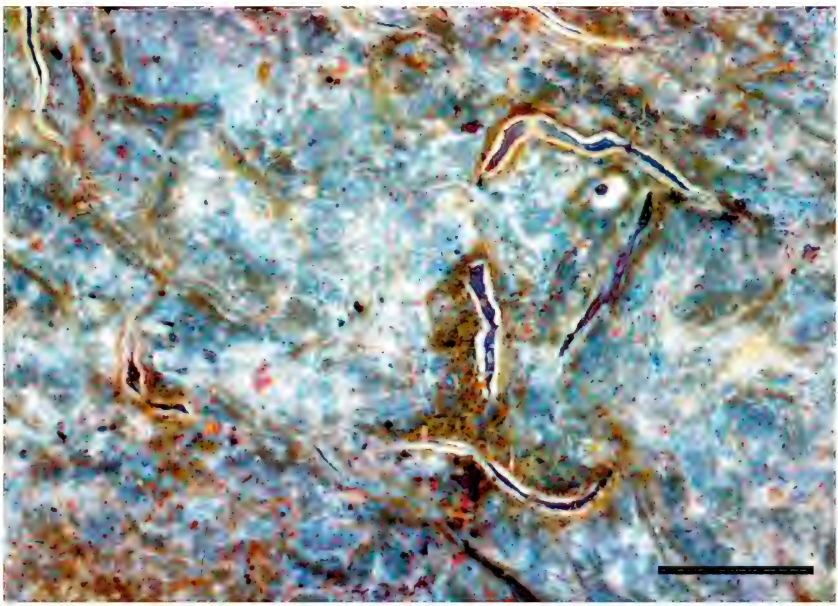

PLATE.2. *Fissurina dumastioides*. Habit. Scale bar: 2 mm

CHEMISTRY: K+ yellow, PD+ yellow-orange, C–; stictic acid detected in TLC.

DISTRIBUTION & ECOLOGY: The species is known to occur in Puerto Rico and India (Fink 1927, Sharma et al. 2012), and has now been identified from Vietnam. It was collected from trees in an evergreen tropical forest of the Chu Yang Sin National Park at an altitude between 700–800 m.

SPECIMEN EXAMINED: **VIETNAM. DAK LAK PROVINCE:** Chu Yang Sin National Park, 12°27′57.0″N 108°20′34.9″E, alt. ca. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nguyen 120267-1 (KoLRI).

REMARKS: *Fissurina dumastioides* resembles *F. dumastii* morphologically and anatomically, but differs in containing stictic acid as thallus compound.

Fissurina egena (Nyl.) Nyl., Sert. Lich. Trop.: 13, 1891

This taxon is morphologically similar to *F. cingalina* (Nyl.) Staiger, with a ochre, yellow-grey, yellowish green, slightly rough or warty thallus (Staiger 2002). However, *F. egena* can be distinguished from *F. cingalina* in its immersed lirellae with roof-like margins and concealed to slightly exposed disc, its 8-spored asci, its muriform, amyloid, 15–23 × 7–10 µm ascospores and the lack of secondary metabolites.

It has been reported from Singapore, North and South America, and Vietnam (Staiger 2002; Aptroot & Sparrius 2006).

Fissurina elaiocarpa (A.W. Archer) A.W. Archer, Telopea 11: 71, 2005.
= *Fissurina marginata* Staiger, Bibliotheca Lichenologica 85: 144, 2002.

This species is characterized by a olive green, slightly subtuberculate, glossy thallus, conspicuous, sessile, straight, curved or sinuous, unbranched, ca. 1–8 × 4 mm ascomata, scarcely open lip, reddish brown proper exciple, 150–200 µm high hymenium, 4–5 × 3–4 locular, 21–28 × 13–18 µm large, amyloid ascospores and absence of secondary metabolites (Archer 2009).

Aptroot & Sparrius (2006) reported this species from Vietnam as *F. marginata* and collected it from a secondary mountain forest of Ba Vi National Park (Hanoi Province), at a 1200–1300 m altitude. The species has a worldwide distribution and is found in Africa, Australia, South and Western America, and Western Indian Ocean (Staiger 2002).

Fissurina incrustans Fée, Essai Crypt. Écorc.: 60, 1825.

This species is characterized by a beige green, smooth to warty thallus, elongate to short (1–4 × 0.3–0.4 mm), sinuous, branched ascomata, erumpent labia, gaping disc, poorly developed proper exciple, usually intermingled with bark substratum, 8-spored asci, muriform, amyloid ascospores of 15–28 × 7–13 µm and absence of secondary compounds. It closely resembles *F. egena* in having similar-sized muriform amyloid ascospores and a thallus lacking chemical compounds, but *F. incrustans* has erumpent lirellae more similar to the ones found in *Platythecium* than the ones found in *Fissurina* (Staiger 2002; Lücking et al. 2011).

The species has been reported in Vietnam from a secondary mountain forest in Ba Vi National Park by Aptroot & Sparrius (2006). It is also found in South America (Staiger 2002).

Fissurina instabilis (Nyl.) Nyl., Sert. Lich. Trop.: 36, 1891. Pl. 3

Thallus corticolous, epiphloeodal, corticate, ≤300 µm thick, glossy, smooth, continuous to ± verrucose (due to bark texture), non-rimose, green, olive green to pale green; cortex 35–45 µm thick; algal layer well developed, continuous, densely integrated with crystals, ≤215 µm thick; medulla indistinct, mostly endophloeodal, integrated with crystals; calcium oxalate crystals small to moderately large, clustered or scattered; prothallus indistinctly black. Vegetative propagules not seen.

Ascocarps numerous, lirelliform, inconspicuous, immersed to ± erumpent, indicated by a thin line between two lips (labia), sometimes slightly open, slightly raised, curved or sinuous, often branched, ≤10 × 0.6 mm, *globulifica*-type (Staiger 2002); labia entire, paler than the thallus; disc slit-like, narrow, scarcely open in mature apothecia, flesh-colored. Thalline margin concolorous with the thallus, thick, ±incurved or straight, 180–215 µm thick. Proper

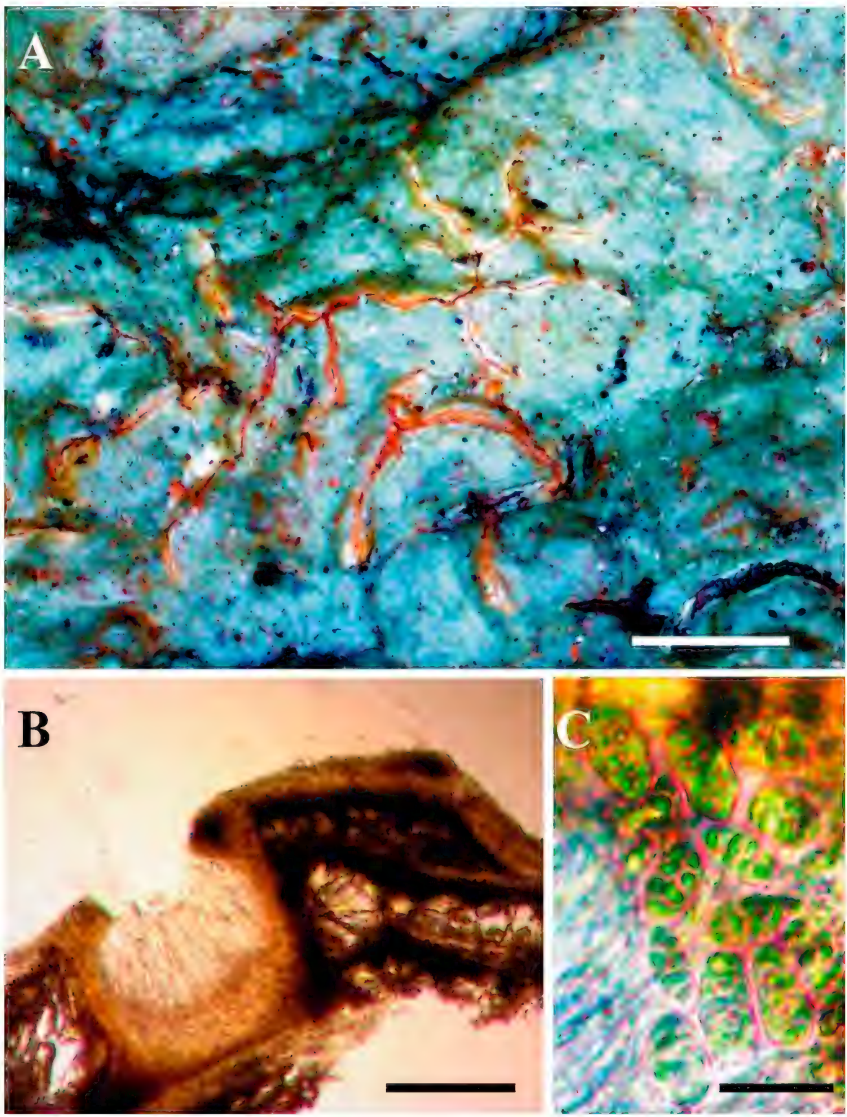

Plate 3. *Fissurina instabilis* A. Habit. B. Cross section of apothecia. C. Ascospores. Scale bars: A = 2 mm; B = 100 µm; C = 20 µm.

exciple well developed, brownish to reddish brown, superficially exposed as a red-brown lining, ca. 30–55 µm thick. Epihymenium hyaline, indistinct, with greyish or brownish granules, 3–6 µm high. Hymenium hyaline, clear, to ca. 75–100 µm high, I–. Paraphyses straight to bent, ±interwoven, unbranched,

with slightly thickened tips, moderately to distinctly conglutinated, 1–2 µm thick. Subhymenium distinct, hyaline, ca. 40–50 µm high. Asci clavate, 8-spored, ca. 65–80 µm, I–. Ascospores uni- or biseriate, hyaline, muriform, oblong to ellipsoidal, with ± rounded ends, thick walled, 13–20 × 7–11 µm, with 3–4(–5) × 1–2 ±round locules, I+ blue violet.

Chemistry: K–, PD–, C–; no lichen compound detected in TLC.

Distribution & ecology: This species has a Pacific distribution (Staiger 2002). In Vietnam it was collected from tree trunks in an evergreen forest of the Chu Yang Sin National Park at an altitude between 700–800 m.

Specimens examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°27′57.0″N 108°20′34.9″E, alt. ca. 780 m, on tree trunk, 21 April 2012, Hur, Oh & Nguyen 120132 (KoLRI); 12°28′12.3″N 108°20′59.9″E, alt. ca. 763 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen 120087 (KoLRI).

Remarks: *Fissurina instabilis* resembles *F. confusa* Common & Lücking and *F. globulifica* (Nyl.) Staiger in having the same thallus morphology, *globulifica*-type lirellae, and muriform amyloid ascospores but *F. confusa* and *F. globulifica* produce psoromic acid and *F. globulifica* has smaller globose (10 × 10 µm) ascospores (Lücking et al. 2011).

Fissurina radiata Mont., Annls Sci. Nat., Bot., sér. 2, 18: 280, 1842.

This species differs from other *Fissurina* species in having aggregated, mostly radiate ascomata forming a pseudostroma, inconspicuous and gaping labia, amyloid, 4-locular ascospores of 15 × 7 µm, and a thallus lacking secondary metabolites (Lücking et al. 2011). It is comparable to material described from Florida as *Fissurina aggregatula* Common & Lücking (Lücking et al. 2011), which differs in having conspicuous labia and a concealed disc, sparsely branched aggregated lirellae, and non-amyloid ascospores.

Fissurina radiata has been reported from the Botanical Garden (Bach Thao Park) in Hanoi City in Vietnam at an altitude between 50–60 m (Aptroot & Sparrius 2006). It has a distribution in tropical regions of America (Lücking et al. 2011), Malaysia, Singapore, and Taiwan.

Fissurina rubiginosa (Fée) Staiger, Biblioth. Lichenol. 85: 148, 2002. Pl. 4

Thallus corticolous, epiphloeodal, corticate, ≤350 µm thick, glossy, smooth, continuous, non-rimose, green, olive green to pale green; cortex 30–50 µm thick; algal layer well developed, continuous, densely integrated with crystals, ≤200 µm thick; medulla indistinct, mostly endophloeodal, studded with crystals; calcium oxalate crystals small to moderately large, clustered or scattered; prothallus indistinctly black. Vegetative propagules not seen.

Ascocarps numerous, scattered, lirelliform, inconspicuous, immersed, indicated by a thin line, sometimes slightly open, slightly raised and paler than

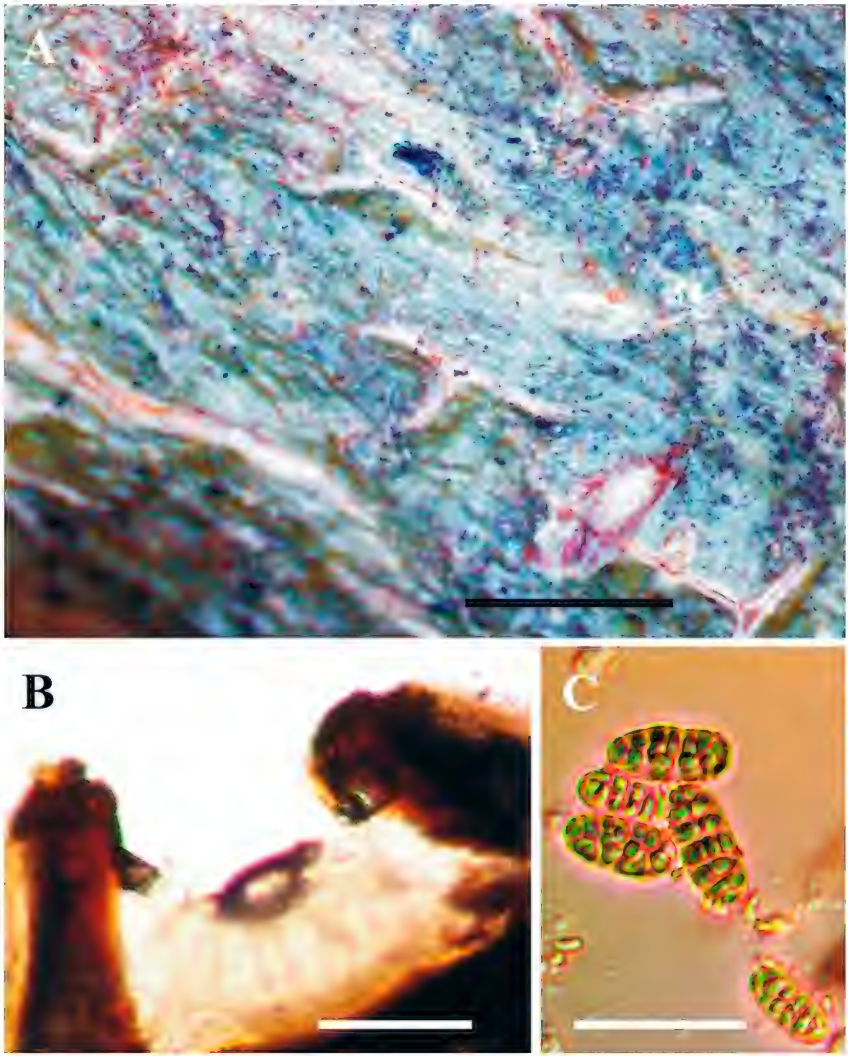

Plate 4. *Fissurina rubiginosa*. A. Habit. B. Cross section of apothecia. C. Ascospores. Scale bars: A = 2 mm; B = 100 µm; C = 30 µm.

the thallus, straight, curved or sinuous, often branched, ≤2 × 0.25 mm, *dumastii*-type; disc slit-like, narrow, scarcely open in mature apothecia, flesh-colored. Thalline margin concolorous with thallus, thick, ±incurved or straight, 75–150 µm thick. Proper exciple well developed, pale to reddish brown, intermingled with bark cells, ≤75 µm thick. Epihymenium hyaline, indistinct, with greyish

or brownish granules, 10–15 µm high. Hymenium hyaline, clear, ≤120–150 µm high, I–. Paraphyses straight to bent, ±interwoven, unbranched, with slightly thickened tips, moderately to distinctly conglutinated, 1–2 µm thick. Subhymenium indistinct, hyaline, ca. 25–30 µm high. Asci clavate, 8-spored, ca. 65–80 µm, I–. Ascospores uni- or biseriate, hyaline, muriform, oblong to ellipsoidal, with ±rounded ends, thick walled, 20–27 × 10–12 µm, with 6–8 × 2–3 ± round locules, I–.

Chemistry: K–, PD–, C–; no lichen compound detected in TLC.

Distribution & ecology: The species is found in French Guiana, USA and India (Sharma et al. 2012), but is now also reported from Vietnam where it was and collected from tree trunks in an evergreen forest of the Chu Yang Sin National Park at an altitude between 700–800 m.

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′12.3″N 108°20′59.9″E, alt. ca. 763 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen 120246 (KoLRI).

Remarks: This species is close to *F. elaiocarpa*. Their thallus morphology is similar and they both have muriform ascospores, although the spores are comparatively larger (18–32 × 13–19 µm) and I+ blue in *F. elaiocarpa*. Another closely related taxon, *Fissurina* aff. *elaiocarpa*, resembles *F. rubiginosa* in having muriform, I– ascospores of similar size (25 × 15 µm), but differs in labiate ascomata and verrucose thallus. The description above does not agree with that of *F. rubiginosa* provided by Sharma et al. (2012), which describes a brown, cracked and rough thallus and much smaller lirellae (0.4–0.6 mm). However, the investigated sample shares some main characteristics with *F. rubiginosa*, such as *dumastii*-type lirellae and non-amyloid ascospores. Because of insufficient material, we provisionally identify our specimen from Vietnam as *F. rubiginosa*.

Fissurina undulata (Müll. Arg.) M. Nakan. & Kashiw., Bull. natn. Sci. Mus., Tokyo, B 29: 87, 2003. Pl. 5

Thallus corticolous, epiphloeodal, corticate, to ca. 400 µm thick, glossy, verrucose to smooth, continuous, non-rimose or slightly cracked due to bark texture, green, olive green to pale green or dull green; cortex 25–45 µm thick; algal layer well developed, continuous, densely integrated with crystals, ≤100 µm thick; calcium oxalate crystals small to moderately large, clustered or scattered; medulla indistinct, mostly endophloeodal, studded with crystals; prothallus not seen. Vegetative propagules not seen.

Ascocarps numerous, scattered, lirelliform, inconspicuous, immersed, indicated by a thin line, sometimes slightly open, slightly raised and paler than the thallus, straight, curved or sinuous, often branched, ≤2 × 0.3 mm, *dumastii*-type; disc slit-like, narrow, scarcely open in mature apothecia, flesh-

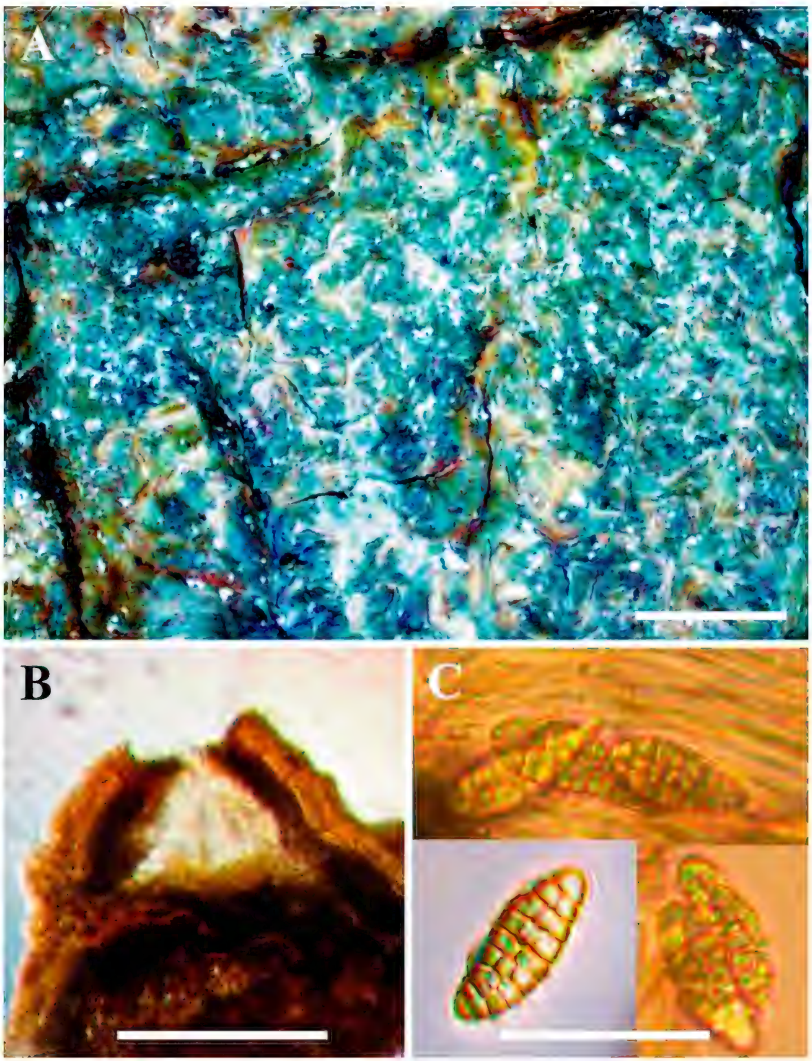

Plate 5. *Fissurina undulata*. A. Habit. B. Cross section of apothecia. C. Ascospores. Scale bars: A = 2 mm; B = 200 µm; C = 30 µm.

colored. Thalline margin concolorous with thallus, thick, ±incurved or straight, 50–100 µm thick. Proper exciple poorly to well developed in mature fruits, hyaline to pale, reddish brown when intermingled with bark cells, ≤30 µm thick. Epihymenium hyaline, indistinct, with greyish or brownish granules. Hymenium hyaline, clear, to ca. 60–100 µm high, I–. Paraphyses straight to bent, ±interwoven, unbranched, with slightly thickened tips, moderately to

distinctly conglutinated, 1–2 µm thick. Subhymenium indistinct, hyaline, ca. 20–30 µm high. Asci clavate, 8-spored, ca. 70–95 × 12–15 µm, I–. Ascospores uni- or biseriate, hyaline, muriform, oblong to ellipsoidal, with ±rounded ends, thick walled, gelatinous sheath present, 22–32(–35) × 10–13 µm, with 6–12 × 2–4 locules, I–; locules ±round to angular.

Chemistry: K–, PD–, C–; no lichen compounds detected in TLC.

Distribution & ecology: The species is found in Japan (Nakanishi 1966), but is now also reported from Vietnam where it was collected from tree trunks in an evergreen forest in Chu Yang Sin National Park at an altitude between 700–800 m.

Specimen examined: **VIETNAM. Dak Lak province:** Chu Yang Sin National Park, 12°28′12.3″N 108°20′59.9″E, alt. ca. 763 m, on tree trunk, 20 April 2012, Hur, Oh & Nguyen 120247 (KoLRI).

Remarks: The above description is based on a specimen with mostly immature fruits, which appear more similar to *F. undulata* than to *F. inabensis* (Vain.) M. Nakan. & Kashiw. (erroneously cited as *F. fujisanensis* (Kashiw. & M. Nakan.) M. Nakan. & Kashiw. in Nakanishi et al. 2003). Both *F. undulata* and *F. inabensis* produce stictic acid and have similar-sized ascospores (21–30 × 10–14 µm for *F. inabensis*). However, a thin thallus, a well developed proper exciple, crenate labia, a medulla with gonidia and 2–3-celled ascospores clearly separate *F. inabensis* from *F. undulata* (Nakanishi 1966). Although the descriptions of *F. undulata* by Nakanishi (1966), Nakanishi et al. (2003), and Sharma et al. (2012) differ in the number of ascospores per ascus (8-spored asci sensu Nakanishi vs. 1-spored asci sensu Sharma), our material agrees well with a species with 8-spored asci. Moreover, the ascospore size (30–38 × 15–18 µm) of *F. undulata* is in conflict with the ascospore size of species with 1-spored asci. A further revision of *F. undulata* would be necessary, as it seems closely related to *F. inabensis*, particularly in immature stages (Nakanishi 1966). Pending some more collections, the Vietnamese material is placed in *F. undulata*.

Key to *Fissurina* species recorded from Vietnam

1a. Thallus containing stictic acid 2
1b. Thallus lacking secondary metabolites 3
2a. Ascospores transversely septate ..... ***F. dumastioides***
2b. Ascospores muriform ..... ***F. rubiginosa***
3a. Ascospores transversely septate 4
3b. Ascospores muriform 5
4a. Lirellae mostly aggregated, radiate, forming a pseudostroma ..... ***F. radiata***
4b. Lirellae disperse, solitary, branched, non-radiate ..... ***F. dumastii***
5a. Ascospores non-amyloid, 22–32(–35) × 10–13 µm ..... ***F. undulata***
5b. Ascospores distinctly amyloid 6

6a. Lirellae immersed, fissurine, roof like margins, ascospores 15–23 × 7–10 µm ..... ***F. egena***

6b. Lirellae immersed to erumpent or prominent, labiate.....7

7a. Lirellae immersed to ± erumpent, ascospores 13–20 × 7–11 µm ..... ***F. instabilis***

7b. Lirellae erumpent to prominent 8

8a. Thallus ± smooth, proper exciple poorly developed, ascospores 15–28 × 7–13 µm ..... ***F. incrustans***

8b. Thallus verrucose, proper exciple well developed, ascospores 21–28 × 13–18 µm ..... ***F. elaiocarpa***

Acknowledgments

This work was supported by a grant from the National Research Foundation of Korea (#2011-0031494) and the Korea National Research Resource Center Program. Authors are grateful to Dr. Robert Lücking, Dr. László Lőkös, and Dr. Cecile Gueidan for their valuable comments on the manuscript. Santosh Joshi also thanks Dr André Aptroot for providing the lichen checklist of Vietnam, and helpful suggestions.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.323

Volume 124, pp. 323–332 *April–June 2013*

Studies of North American macrofungi, 1. Validation of *Lactarius rubidus* comb. nov. and *Leccinellum quercophilum* sp. nov.

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Abstract — Two mycorrhizal taxa are described: *Lactarius rubidus* from the west coast and *Leccinellum quercophilum* from eastern North America. *Lactarius rubidus* is a new combination for *Lactarius fragilis* var. *rubidus,* here validated after a previous invalid publication. *Leccinellum quercophilum* is a new species. Detailed macro- and microscopic descriptions, ITS and LSU sequences, illustrations of microscopic features, and plates are presented for each species; a type study of *Lactarius fragilis* var. *rubidus* is also provided.

Key words — *Boletaceae, Boletales, Russulaceae, Russulales*, taxonomy

Introduction

The North American macrofungi known as *Lactarius fragilis* var. *rubidus* and *Leccinum griseum* (also sometimes known as *Leccinum carpini*) are commonly collected fungi for which currently applied names are unsatisfactory. The west coast fungus *Lactarius fragilis* var. *rubidus,* which differs substantially from the eastern North American type variety in morphology, ecology, and geographic distribution, merits taxonomic rank as a species. *Leccinum griseum* is a European fungus clearly distinct from the North American entity frequently bearing its name in herbaria. Here we describe *Leccinellum quercophilum*, a North American bolete sometimes identified as *Leccinum griseum,* as a new species and validate the combination *Lactarius rubidus* to apply to *Lactarius fragilis* var. *rubidus*.

Materials & methods

Both fresh basidiomes and herbarium specimens were studied. Colors were recorded and codified following Kornerup & Wanscher (1967), or the Online Auction Color Chart (www.onlineauctioncolorchart.com — abbreviated "OAC" herein). Microscopic features were studied using hand sections of fresh material, and of dried specimens rehydrated in water after immersion in 90% alcohol. Sections were mounted in 2% KOH and Melzer's reagent, and viewed using an Olympus BX51 microscope. Specimens are deposited in herbaria and cited according to Thiers (2012).

DNA from each taxon was extracted from small hymenium-bearing pieces of dried basidiocarp following the methods of Lindner & Banik (2009). Protocols for ITS amplification, sequencing, sequence editing, and other related procedures followed Lorch et al. (2013) with the following modifications: denaturing for 40 s, annealing at 53 °C for 40 s, and extension for 90 s in the 30 PCR cycles. LSU sequences were obtained with the same protocols using the primers LROR (Moncalvo et al. 2000) and either LR5 or LR7 (Vilgalys & Hester 1990). Newly generated DNA sequences were deposited in GenBank and compared with available sequences via GenBank BLAST searches.

Taxonomy

Lactarius rubidus (Hesler & A.H. Sm.) Methven, **comb. & stat. nov.** PLATE 1

MYCOBANK MB803100

≡ *Lactarius fragilis* var. *rubidus* Hesler & A.H. Sm., N. Amer. Sp. *Lactarius*: 505. 1979.

"*Lactarius rubidus*" Methven, *Agaricales* (Gilled Fungi) of California, 10, II: 67. 1997, nom. inval. [provisional name].

PILEUS 2–8 cm broad, convex to plane, disc shallowly depressed, occasionally umbonate; margin incurved to decurved, glabrous; surface glabrous, dry to moist, rugose to rugulose, azonate, light brown (6D7-5) to brown (7D8-5), or reddish brown (8D8-6), negative with the application of 15% KOH; context up to 5 mm thick at the disc, light orange (5A4-2), unstaining on exposure; odor of maple syrup or fenugreek when dried; taste not distinctive. LAMELLAE adnate to subdecurrent, close to distant, narrow, rarely forked, light orange (5A4-2, 6A4-3), not marginate, unstaining where cut. STIPE 2–7 cm × 4–10(–15) mm, terete, equal; surface glabrous, dry, not scrobiculate, brownish orange (6C7-5) to light brown (7D7-5); white to light orange tomentum at the base; context hollow, concolorous with the pileal context, unstaining on exposure. LATEX whey-like, not copious, unstaining, unchanging, taste not distinctive.

BASIDIOSPORES white to pale yellow (4A3-2) in mass, 6–8(–8.5) × 6–7.5 µm, globose to subglobose; amyloid ornamentation a broken to partial or nearly complete reticulum 0.5–1 µm high. BASIDIA 35–55 × 7.5–12.5 µm, tetrasterigmate. CHEILOCYSTIDIA 25–35 × 6–9 µm, clavate. MACROCYSTIDIA not observed. PILEIPELLIS an epithelium composed of clavate to vesiculose cells in short chains, tangled in age, dry. PILEUS TRAMA heteromerous, sphaerocysts in rosettes, lactiferous hyphae inconspicuous. STIPITIPELLIS a simple cutis with

PLATE 1: *Lactarius rubidus*, M. Kuo 01131106 (NY). Basidiocarps (top), basidiospores (middle), and pileipellis (bottom). Scale bars = 10 µm. [For additional illustrations see Hesler & Smith (1979: Fig. 247, basidiospores) and Methven (1997: Pl. 2, basidiocarps).]

scattered projecting hyphal tips, stipe cortex heteromerous, sphaerocysts in rosettes.

ECOLOGY & DISTRIBUTION — Scattered, gregarious, or caespitose in duff, in coastal coniferous-deciduous forests in apparent association with *Quercus agrifolia, Notholithocarpus densiflorus,* and *Pseudotsuga menziesii.* Common. September through February. California, Oregon, and Washington.

COLLECTIONS EXAMINED — **UNITED STATES. CALIFORNIA**: MARIN COUNTY, 10 Dec 1982, ASM 2261 (EIU); 19 Dec 1984, ASM 3523 (EIU); 27 Dec 1984, ASM 3632

(EIU). Mendocino County, 30 Apr 1983, CM Bern, ASM 2456 (EIU); 13 Dec 1990, ASM 6523 (EIU). San Mateo County, 17 Dec 1984, ASM 3600 (EIU); 12 Jan 1985, H Saylor 2297 (EIU); 13 Jan 2005, M Kuo 01130522 (NY); 13 Jan 2011, M Kuo 01131106 (NY, GenBank ITS KC691205, LSU KC691206). Sonoma County, 02 Feb 2003, M Kuo 02200309 (NY). **Oregon**: Polk County, 14 Nov 1970, AH Smith 79939 (MICH 23242, paratype); 14 Nov 1970, AH Smith 79942 (MICH 11133, holotype).

Comments — *Lactarius rubidus* is characterized by the reddish brown, azonate, dry to moist pileus, whey-like latex, the maple syrup or fenugreek odor when dried, and globose basidiospores with a broken to partial or nearly complete amyloid reticulum. *Lactarius fragilis* (Burl.) Hesler & A.H. Sm. var. *fragilis*, known from eastern North America, differs in having a snuff brown to burnt umber pileus, subdistant yellowish lamellae, and globose basidiospores with heavier deposits of amyloid ornamentation that form a partial to complete reticulum. *Lactarius rubidus* has frequently been misidentified as *L. camphoratus* (Bull.) Fr. However, *L. camphoratus* is a European and eastern North American species featuring a red brown pileus, close to crowded pale pinkish cinnamon lamellae, milk-white latex, and broadly ellipsoid basidiospores with amyloid ornamentation of isolated spines and nodulose ridges forming at most a broken reticulum. *Lactarius rubidus* is a popular edible commonly called the "candy cap" by collectors (Kuo 2007). Wood et al. (2012) recently determined quabalactone III to be the source of the maple syrup odor of *L. rubidus*.

ITS sequence comparisons via GenBank BLAST searches indicate *L. rubidus* is not conspecific with European and North American *L. camphoratus*. *Lactarius* cf. *rubidus* (GenBank DQ822820.1 from a basidiocarp and GU180303.1 from a *Pinus muricata* root tip; Peay et al. 2007) and unidentified samples GenBank AF323116.1 from a *Pinus muricata* root (Wurzberger et al. 2001) and DQ273391.1 from a *Notholithocarpus densiflorus* root (Bergemann & Garbelotto 2006), all from California, appear to represent *L. rubidus* at 99% sequence identity.

In Methven (1997: 67), the combination "*Lactarius rubidus* (Hesler & A.H. Sm.) Methven" appeared with a citation of its forthcoming publication as "Mycotaxon (in press). 1998" but was not validly published since it was merely proposed in anticipation of future acceptance of this taxon at the species rank (ICN Art. 36.1, McNeill et al. 2012). The subsequent Mycotaxon publication did not occur, and the combination *Lactarius rubidus* has not been validly published elsewhere since. A record for "*Lactarius rubidus* Arora 1991" appears in Index Fungorum (www.indexfungorum.org) but no further information is provided; a similar record appears in the MycoBank database (www.mycobank.org), again without any supporting publication information. David Arora's (1991) field guide, All That the Rain Promises and More: a Hip Pocket Guide to Western Mushrooms is the only plausible basis we have been able to discover for these website entries. Since Arora treats the species as "*Lactarius fragilis*," listing

"other names" as "*Lactarius fragilis* var. *rubidus, L. rubidus*," without citing taxon authorities or providing full and direct references to any of the names as required for publishing a new combination, his book cannot be considered a valid publication of *Lactarius rubidus*. We validate the combination here.

Study of the Holotype — The holotype of *Lactarius fragilis* var. *rubidus* is held in the University of Michigan Herbarium (MICH 11133) and was collected by A.H. Smith (79942) on "rotten wood" in the Van Duzer Corridor, Polk County, OR; it consists of several well preserved basidiomes with a strong odor of fenugreek. Basidiospores 6.5–8.5 × 6–7.5 µm, globose to subglobose, ornamentation a partial to more or less complete reticulum 0.5–1 µm high, with scattered free ends and rare isolated elements, plage conspicuous and lacking amyloid debris. Basidia 35–50 × 7.5–12.5 µm, clavate, tetrasterigmate. Cheilocystidia not clearly differentiated. Macrocystidia not observed. Pileipellis an epithelium composed of vesiculose or irregularly shaped inflated cells in short chains, at times aggregated into mounds, especially in age. Pileus Trama heteromerous. Stipitipellis a dry, simple cutis of interwoven hyphae with scattered, projecting, pyriform to subfusiform hyphal tips 25–35 × 7.5–15 µm.

Leccinellum quercophilum M. Kuo, **sp. nov.** Plate 2

MycoBank MB802407

Differs from *Leccinum griseum* by its bluish green staining on the pileus and stipe, its creamy whitish tubes, its context staining gray when sliced, and its association with *Quercus* in eastern North America.

Type: United States, Illinois, Coles County, Charleston, under *Quercus alba* on a lawn, 12 July 2008, coll. M Kuo 07120801 (**Holotype**, NY; GenBank ITS KC691207, LSU KC691208).

Etymology: from a combination of the Latin *Quercus* = oak, and the Greek φιλος = friend; "oak-loving."

Pileus 3–9 cm broad; convex; dry; glabrous; dull orangish brown (OAC 749) to medium yellow-brown (OAC 707, 715); rugulose-pitted when young, becoming conspicuously areolate; without an overlapping sterile margin; discoloring bluish green marginally with age; surface negative with the application of ammonia or $FeSO_4$, negative to yellowish with 15% KOH. Hymenophore tubulose; depressed at the stipe. Tubes 1–2 cm deep, creamy whitish (contrasting with the pore surface), discoloring slowly blackish when sliced; pores 1–3 per mm at maturity, round becoming angular with maturity, whitish or medium grayish brown (OAC 736) when young, becoming yellowish brown to brownish; discoloring greenish in places with age; bruising slowly dark brown, with or without a bluish stage. Stipe 5–9 cm x 8–20 mm; equal, or tapered slightly to apex, or slightly ventricose; dry; whitish underneath fine, tiny scabers that are whitish apically and brown below; the scabers arranged in vague longitudinal

lines, darkening somewhat with age but not blackening; bruising or staining greenish to bluish basally; basal mycelium whitish. CONTEXT whitish, staining grayish to gray within 15–30 minutes of being sliced, with or without a faintly pinkish stage; sometimes staining bluish in the stem base when sliced; pinkish to negative with the application of ammonia, negative to greenish with $FeSO_4$, gray to greenish gray with 15% KOH. ODOR AND TASTE not distinctive.

BASIDIOSPORES 15–18(–28) × 5–7.5 µm; fusiform; ochraceous in 2% KOH; inamyloid; aseptate; smooth. BASIDIA 25–30 × 9–12 µm; clavate; tetrasterigmate. PLEUROCYSTIDIA 25–40 × 8–12 µm; occasional or frequent; fusoid-ventricose; thin-walled; smooth; hyaline to ochraceous or golden in 2% KOH. CHEILOCYSTIDIA 35–50 × 5–10 µm; fusoid-ventricose to mucronate, subclavate, or irregular; thin-walled; smooth or roughened; ochraceous to golden in 2% KOH. CAULOCYSTIDIA 30–50 × 10–15 µm; mostly fusoid-ventricose but occasionally ventricose-rostrate, or lageniform; apices occasionally mucronate; thin-walled; smooth; hyaline to ochraceous in 2% KOH. PILEIPELLIS a trichodermium of septate hyphae; hyaline to ochraceous or brownish in 2% KOH; terminal elements subglobose to clavate or irregular, 25–50 µm wide.

ECOLOGY & DISTRIBUTION — Scattered to gregarious under oaks; appearing in hardwood forests and in urban settings (lawns, cemeteries, etc.); summer and early fall; currently known from Illinois and Michigan.

ADDITIONAL COLLECTIONS EXAMINED — **UNITED STATES. ILLINOIS:** COLES COUNTY, 20 Jun 2010, M Kuo 06201003 (NY). **MICHIGAN:** LIVINGSTON COUNTY, Aug 1972, AH Smith 84011 (MICH 35770, as *Leccinum griseum*); WASHTENAW COUNTY, 16 Sep 1940, AH Smith 15410 (MICH 35774, as *Leccinum griseum*).

COMMENTS — *Leccinellum quercophilum* can be identified by its association with oaks, the dull brown or yellowish brown rugulose-pitted cap that becomes conspicuously areolate, the fine brown scabers, the green and blue staining, and the trichoderm pileipellis. It has been thought to represent the European species *Leccinum griseum* (Quél.) Singer (≡*Gyroporus griseus* Quél.) by various North American authors, including Smith & Thiers (1971: 214). However, the name *Leccinum griseum* is problematic (Lannoy & Estades 1995; den Bakker & Noordeloos 2005). Regardless of its correct name, this European species, sometimes listed as *Leccinum carpini* (R. Schulz) D.A. Reid (Lannoy & Estades 1995) or *Leccinum pseudoscabrum* (Kallenb.) Šutara (den Bakker & Noordeloos 2005), is primarily associated with *Carpinus* and does not stain blue or green (except very rarely in the base of the stem). Also, the European species has yellowish tubes when young and flesh that stains deep violet when sliced (Lannoy & Estades 1995; den Bakker & Noordeloos 2005)—a difference noted by Smith & Thiers (1971). On the basis of these morphological differences, we recognize the North American species as new and have selected an epithet reflecting its association with oaks.

Plate 2: *Leccinellum quercophilum*, M. Kuo 07120801 (NY; holotype). Basidiocarps (top), pileipellis elements (bottom left), cheilocystidia (middle right), and basidiospores (bottom right). Scale bars = 10 µm.

Leccinellum Bresinsky & Manfr. Binder was erected in 2003 (Bresinsky & Besl 2003) to accommodate *Leccinum* species with a yellow hymenophore and a trichoderm-like pileipellis. *Boletus nigrescens* Richon & Roze (nom. illegit.; ≡ *Leccinellum nigrescens* (Singer) Bresinsky & Manfr. Binder) was designated as the type of the genus. Bresinsky & Besl (2003) included *Leccinum carpini* and *L. griseum* among eight existing species they accepted in *Leccinellum*, but the nomenclature and taxonomy warrant review. Although the hymenophore of *Leccinellum quercophilum* is not yellow, it falls within *Leccinellum* as defined in Bresinsky & Besl (2003) on the basis of its pileipellis.

GenBank BLAST searches with an ITS sequence of *Leccinellum quercophilum* reveal that the closest matches include *Leccinum carpini* [AF454588.1 (The Netherlands)], *Leccinum crocipodium* (Letell.) Watling [AF454589.1 (Belgium), JN021053.1 (France), JF908325.1 unpublished (Italy), and AF454590.1 (The Netherlands)], and *Leccinum talamancae* Halling et al. [AY544779.1 (Costa Rica)] as labeled and numbered in GenBank with published citations (den Bakker et al. 2004a,b; Dentinger et al. 2011). The >3% difference in sequence identity supports *Leccinellum quercophilum* as a distinct species. Morphological differences also separate these taxa. As noted above, the European species associated with *Carpinus* has yellowish young tubes, flesh that turns violet when sliced, and does not stain blue or green; *Leccinum crocipodium* has a yellow hymenophore and does not stain green (den Bakker & Noordeloos 2005); *Leccinum talamancae* is less areolate and features flesh that turns slowly pink to reddish orange in the pileus and stipe apex when exposed (Halling 1999). Our own additional unpublished alignments and phylogenetic analyses, along with the ITS tree in Lebel et al. (2012a,b), support generic placement in *Leccinellum*. Further research will determine whether *Leccinellum* as presently circumscribed is or is not supported phylogenetically.

Acknowledgments

We are grateful to Timothy J. Baroni and Patrick R. Leacock for serving as pre-submission reviewers and for their helpful comments on the manuscript; Darvin DeShazer also provided helpful commentary. We thank the University of Michigan Herbarium (MICH) for kindly lending specimens, and the William and Linda Steere Herbarium of the New York Botanical Garden (NY) for processing deposits. We also thank Henk den Bakker, Dana Ringuette, Barbara Thiers, Jean Toothman, and Sue Yocum for invaluable assistance in various aspects of this study. The U.S. Forest Service, Northern Research Station, Center for Forest Mycology Research provided funds for DNA sequencing. We thank Kyah Norton of the CFMR for providing expert technical support. Support to REH from the National Science Foundation, via grants DEB #9972018, DEB #0414665, and DEB #1020421, is gratefully acknowledged.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.333

Volume 124, pp. 333–340 *April–June 2013*

Cladonia dunensis sp. nov. from southern Brazil, with notes on the genus in beach dune environments

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Abstract — *Cladonia dunensis* is described. The new species resembles *C. celata* in the corticate and scyphose podetia that are simple at first before branching fastigiately but differs in forming squamulose podetia and producing psoromic acid. Seven *Cladonia* species are reported for the first time from beach dune environments in the coastal plain of Santa Catarina State, southern Brazil.

Key words — *Ascomycota*, *Cladoniaceae*, dimorphic thallus, lichens, taxonomy

Introduction

In the Brazilian coastal areas, sand dune habitats are covered by herbaceous, arbustive and arboreal vegetation types named restinga vegetation (Araujo 1992). The lichenized restinga mycota has been intensively studied in the last decade, primarily within arbustive-arboreal vegetation (e.g., Benatti & Marcelli 2008, 2009; Dal-Forno & Eliasaro 2010 a,b; Donha & Eliasaro 2006; Marcelli & Benatti 2011; Martins & Marcelli 2011). However, there is no information about species that colonize the beach dune environments in Brazil (Marcelli 1998).

Cladonia P. Browne, the largest genus in *Cladoniaceae* with c. 400 species (Stenroos et al. 2002; Burgaz & Ahti 2009), is frequent and abundant in arbustive restinga vegetation (Ahti 2000; Gumboski & Eliasaro 2012a,b), and some species can colonize other adjacent environments.

As a part of a survey of the *Cladoniaceae* in the coastal region of southern Brazil (Gumboski & Eliasaro 2012a,b) we present a first report on *Cladonia* species found in the beach dune environment in Brazil, one of which is new to science. Descriptions, comments and illustrations are presented.

Materials & methods

The specimens were collected in depressions between beach dunes in the Municipality of Imbituba, Santa Catarina State, Brazil (Fig. 1). Specimens were examined using standard stereoscopic (20–50×) and light microscopic (400–1000×) techniques. Sections of primary thallus, podetia, and pycnidia were mounted in water. Chemical constituents were identified by spot tests, under UV light, and thin layer chromatography using solvent system C (Orange et al. 2001) and by comparison with authentic samples.

Taxonomy

Cladonia dunensis Gumboski, Beilke & Eliasaro, **sp. nov.** Fig. 2

MycoBank MB 804907

Differs from *Cladonia celata* by its squamulose podetia and by its production of psoromic acid.

Type: Brazil. Santa Catarina State: Municipality of Imbituba, 28°17′57″S 48°42′14″W, sea level, on acid soil in depressions between dunes, 19 Feb 2010, E. Gumboski & F. Beilke 1750 (**Holotype**, UPCB; **isotypes**, ICN, JOI).

Etymology: The epithet refers to the habitat of this species.

Primary thallus evanescent, lobate squamules, 1.0–3.0 mm long, 0.5–1.2 mm wide, planes, not divided, smooth margins, without rhizines, esorediate; upper surface greenish to whitish, corticate, smooth, not pruinose, without pycnidia, lower surface white, ecorticate, smoothly arachnoid; cortex 40–70 μm, medulla 110–420 μm. Podetia 0.8–2.0 cm tall, 0.4–0.9 mm thick, greenish to brownish, scyphose, irregularly branching, at first simple then finally fastigiate mainly dichotomously, but with irregular branches common on podetial sides; scyphi sometimes deformed at margin, ≤1.2 mm wide; surface corticate and verrucose, sometimes cracked (some cracks can reveal the white medulla below), without soredia or granules, schizidia and squamules present, lobate squamules ≤0.5 mm long; cortex 20–50(–80) μm, medulla 50–120 μm, hyaline stereome, 130–190 μm, papillate central canal, 270–500 μm wide. Hymenial disks common, globose, apical on podetia, pale brown to brownish, immature, ascospores not seen. Pycnidia rare, brow to blackish, to 0.2 mm wide, slime and conidia not seen.

Chemistry: Thallus K–, C–, KC–, UV–. Psoromic acid [major] and 2'-O-demethylpsoromic acid [minor] detected by TLC.

Ecology and distribution: The new species is known only on sandy soil in depressions between dunes, where it usually grows intermixed with other *Cladonia* species, such as *C. ramulosa*, *C. didyma*, and *C. merochlorophaea*. This area, characterized by acidity and high humidity, develops an herbaceous vegetation that is often used for cattle grazing and is periodically burned, as observed in November 2011; thus the species is endangered.

Additional specimens examined: **BRAZIL. Santa Catarina State:** Municipality of Imbituba, 28°17′57″S 48°42′14″W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1754b (JOI).

FIGURE 1. The studied area: dunes from Municipality of Imbituba, Santa Catarina State, Brazil.

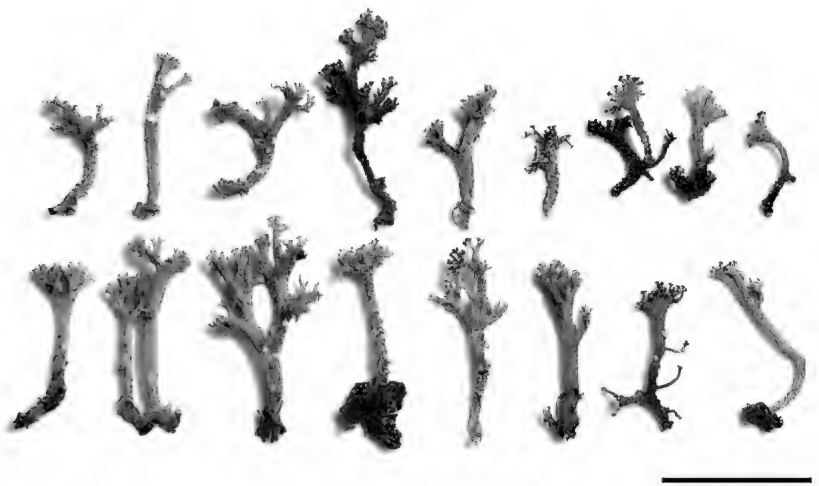

FIGURE 2. *Cladonia dunensis* (holotype). Scale bar = 1 cm.

Remarks: The new species is characterized by the evanescent primary squamules, short, corticate, scyphose and squamulose podetia, at first simple then branching fastigiately, by the brownish ascomata, and the psoromic acid production.

According to the infrageneric classification suggested by Stenroos et al. (2002), *Cladonia dunensis* probably belongs in supergroup *Cladonia* based on its squamulose primary thallus, sparsely branched corticate podetia, unperforated axils, and brownish ascomata.

Cladonia dunensis resembles *C. celata* A.W. Archer in similar podetia (Archer 1984, 1992). However, *C. dunensis* has squamulose podetia with schizidia and contains psoromic acid, while *C. celata* has podetia without schizidia and squamules and produces fumarprotocetraric acid. These two species also exhibit disjunct geographic distributions, with *C. celata* occurring at 450–1100 m in Australia (Archer 1984), whereas *C. dunensis* is known only from the type locality at sea level in dune environments in southern Brazil.

Cladonia fruticulosa Kremp. differs morphologically in having a persistent primary thallus up to 5(–10) mm long and 2(–3) mm wide with crenate-incised to deeply divided squamules, moderately branching podetia with variably smooth or roughly corticate to partly or almost totally sorediate surfaces, and is found at 440–3800 m (Swinscow & Krog 1988; Stenroos 1988).

The specimens of *Cladonia ochracea* found in the studied area are quite similar to *C. dunensis*, but *C. ochracea* has scyphose to capitate podetia, produces fumarprotocetraric acid and grows up to 3.5 cm tall.

Psoromic acid is very rare in *Cladonia* species from Brazil. Gumboski & Eliasaro (2012a,b) recorded 23 species from restinga vegetation and rocky shores from southern Brazil, and none of them produced this compound. Ahti (2000) recorded 180 species from Brazil and psoromic acid was detected in only some specimens of *C. acuminata* (Ach.) Norrl., *C. cartilaginea* Müll. Arg., and *C. dactylota* Tuck. Both *C. acuminata* and *C. cartilaginea* have ascyphose podetia that have a granulose-sorediate surface in *C. acuminata* (Ahti 2000) and are decorticate in *C. cartilaginea* (Herb. ICN, M. Fleig 3416, 6041). In *Cladonia dactylota* the primary thallus is persistent, sometimes sorediate, and the podetia, although scyphose, have sorediate surface with tuberculose soralia below the scyphi or on podetial stalks (Ahti 2000; Herb. ICN, M. Fleig 5191, 5969).

The six other species found in beach dune environments are:

Cladonia crispatula (Nyl.) Ahti, Lichenologist 9: 14. 1977.

Ecological notes: Specimens were found mainly between herbs and isolated from other species on sandy soil. The specimens are smaller and

less frequent when compared to those seen in arbustive restinga vegetation (Gumboski & Eliasaro 2012b).

Specimens examined: **BRAZIL. Santa Catarina State**: Municipality of Imbituba, 28°17′S 48°42′W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1754a, 1755 (JOI); 10 Nov 2011, E. Gumboski, F. Beilke & S. Eliasaro 3020, 3027 (JOI).

Distribution: Occurs from sea level to 2000 m. Recorded only from South America: Brazil, Paraguay and Uruguay (Ahti 2000).

See Ahti (2000), Gumboski & Eliasaro (2012b) for descriptions.

Cladonia didyma (Fée) Vain., Acta Soc. Fauna Fl. Fenn. 4: 137. 1887.

Ecological notes: The most frequent species found in the beach dunes, growing on sandy soil in open sites or between herbs. Usually intermixed with *C. ochracea* and *C. ramulosa* specimens.

Specimens examined: **BRAZIL. Santa Catarina State**: Municipality of Imbituba, 28°17′S 48°42′W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1751 (JOI); 10 Nov 2011, E. Gumboski, F. Beilke & S. Eliasaro 3017, 3022 (JOI).

Distribution: Cosmopolitan, from sea level to 3000 m (Ahti 2000). Recorded from Oceania, Asia (Vainio 1887), Africa (Doidge 1950), North America (Fulford 1937), Central America (Vainio 1887), and South America in Argentina (Calvelo & Liberatore 2002), Bolivia (Ahti 2000), Brazil (Krempelhuber 1876, as *C. muscigena*), Chile (Crombie 1876, as *C. melanodes*), Colombia, Ecuador (Ahti 2000), Guyana (Sipman 1990), and Paraguay, Peru, Suriname, Trinidad & Tobago, Uruguay, Venezuela (Ahti 2000).

See Ahti (2000), Ahti & Hammer (2002), Gumboski & Eliasaro (2012b) for descriptions.

Cladonia latiloba Ahti & Marcelli, Fl. Neotrop., Monogr. 78: 249. 2000.

Ecological notes: Only little thalli of this species were found. The specimens were growing on sandy soil in open sites without vegetation cover around them and usually isolated from other species of *Cladonia*.

Specimens examined: **BRAZIL. Santa Catarina State**: Municipality of Imbituba 28°17′S 48°42′W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1756, 1755 (JOI); 10 Nov 2011, E. Gumboski, F. Beilke & S. Eliasaro 3018, 3019, 3026 (JOI).

Distribution: Recorded only from Brazil, at sea level (Fleig et al. 1995; Ahti 2000).

See Ahti (2000), Gumboski & Eliasaro (2012a) for descriptions.

Cladonia merochlorophaea Asahina, J. Jap. Bot. 16: 713. 1940.

Ecological notes: The specimens were represented by scattered podetia, almost always intermixed with *C. ochracea* and *C. ramulosa* specimens on sandy soil in open places.

Specimens examined: **BRAZIL. Santa Catarina State**: Municipality of Imbituba, 28°17′S 48°42′W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1749 (JOI); 10 Nov 2011, E. Gumboski, F. Beilke & S. Eliasaro 3021, 3025 (JOI).

Distribution: According to Ahti (2000), this species usually occurs in high altitudes from 900 to 4100 m. Recorded from Oceania (Galloway 1985), Asia (Ahti 1976), Europe (Asahina 1940), North America (Archer 1992), and South America in Brazil (Fleig et al. 1995), Colombia (Flakus et al. 2008), Peru, and Venezuela (Ahti 2000).

See Ahti (2000), Burgaz & Ahti (2009), Gumboski & Eliasaro (2012a) for descriptions.

Cladonia ochracea L. Scriba, Cladon. Exs.: no. 1006. 1923.

Ecological notes: Usually growing intermixed with *C. didyma* and *C. ramulosa* specimens on sandy soil in open places. A few specimens were found growing on decaying logs and on an anthill between herbs.

Specimens examined: **BRAZIL. Santa Catarina State**: Municipality of Imbituba, 28°17′S 48°42′W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1752 (JOI); 10 Nov 2011, E. Gumboski, F. Beilke & S. Eliasaro 3023, 3028 (JOI).

Distribution: Occurs from sea level to 1900 m (Ahti 2000). Recorded only from South America in Argentina (Ahti 2000), Brazil (Osorio & Fleig 1991), and Paraguay and Uruguay (Ahti 2000).

See Fleig et al. (1995), Ahti (2000), Gumboski & Eliasaro (2012a) for descriptions.

Cladonia ramulosa (With.) J.R. Laundon, Lichenologist 16: 225. 1984.

Ecological notes: The specimens were mainly found as scattered podetia usually growing intermixed with *C. didyma* and *C. ochracea* specimens on sandy soil in open places.

Specimens examined: **BRAZIL. Santa Catarina State**: Municipality of Imbituba, 28°17′S 48°42′W, sea level, 19 Feb 2010, E. Gumboski & F. Beilke 1748, 1753 (JOI); 10 Nov 2011, E. Gumboski, F. Beilke & S. Eliasaro 3024 (JOI).

Distribution: Cosmopolitan (Burgaz & Ahti 2009), occurring from sea level to 2000 m (Ahti 2000). Recorded from Oceania, Asia, Europe, Africa, North America, Central America (Vainio 1894, as *C. pityrea*), and South America in Argentina (Ferraro & Ahti 1987), Brazil (Osorio & Fleig 1988), Colombia, Paraguay (Ahti 2000), Peru (Vainio 1894, as *C. pityrea*), and Uruguay and Venezuela (Ahti 2000).

See Ahti (2000), Burgaz & Ahti (2009), Gumboski & Eliasaro (2012a) for descriptions.

Acknowledgments

We are grateful to Dr. Alan W. Archer, Dr. Lidia I. Ferraro, Dr. Soili Stenroos, and Msc. Celso Voos Vieira for providing a valuable bibliography and for discussions with the first author and to the reviewers Dr. Marcelo Pinto Marcelli, Dr. Samuel Hammer, and Dr. Shaun R. Pennycook for all the comments and suggestions. The authors also wish to thank CAPES (Coordenadoria de Aperfeiçoamento do Pessoal do Ensino Superior) for granting a Mastership to Gumboski and to Beilke.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.341

Volume 124, pp. 341–347 *April–June 2013*

Coniolepiota spongodes (*Agaricaceae, Basidiomycota*) in Bangladesh and China

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Abstract — The genus *Coniolepiota* is recorded for the first time from Bangladesh and China. *Coniolepiota spongodes* is characterized by its gray lilac to purple floccose basidiomata and relatively small smooth ellipsoid to oblong basidiospores. A detailed morphological description, photographs and line drawings, with confirmation of the phylogenetic position of newly reported taxon are provided herein.

Key words — *Agaricales*, Asian fungus, ITS-nrLSU, morphology, new record

Introduction

Coniolepiota Vellinga, a genus of family *Agaricaceae* recently described from tropical Asia, harbors a single relatively well-known species, *C. spongodes* (Vellinga et al. 2011). During studies of the higher fungi (Hosen & Ge 2011; Hosen et al. 2013), collections of *C. spongodes* were made for the first time from Bangladesh. A study of the Bangladesh and Chinese materials is provided here.

Materials & methods

Morphological studies

Collections were made along roadsides in forests of *Shorea robusta* Gaertn. (*Dipterocarpaceae*) in Bangladesh, and from tropical China. Fresh specimens were described and photographed in the field. Dried specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China (KUN-HKAS), and in the Herbarium of Agarics Flora, Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh (SHAF).

Microscopic observations were made in 5% aqueous KOH, and structures drawn by free hand. The notations '(n/m/p)' indicate that the measurements were made on 'n' basidiospores from 'm' basidiomata of 'p' collections with a minimum of 20 basidiospores per basidioma. Dimensions of basidiospores are presented in the following form

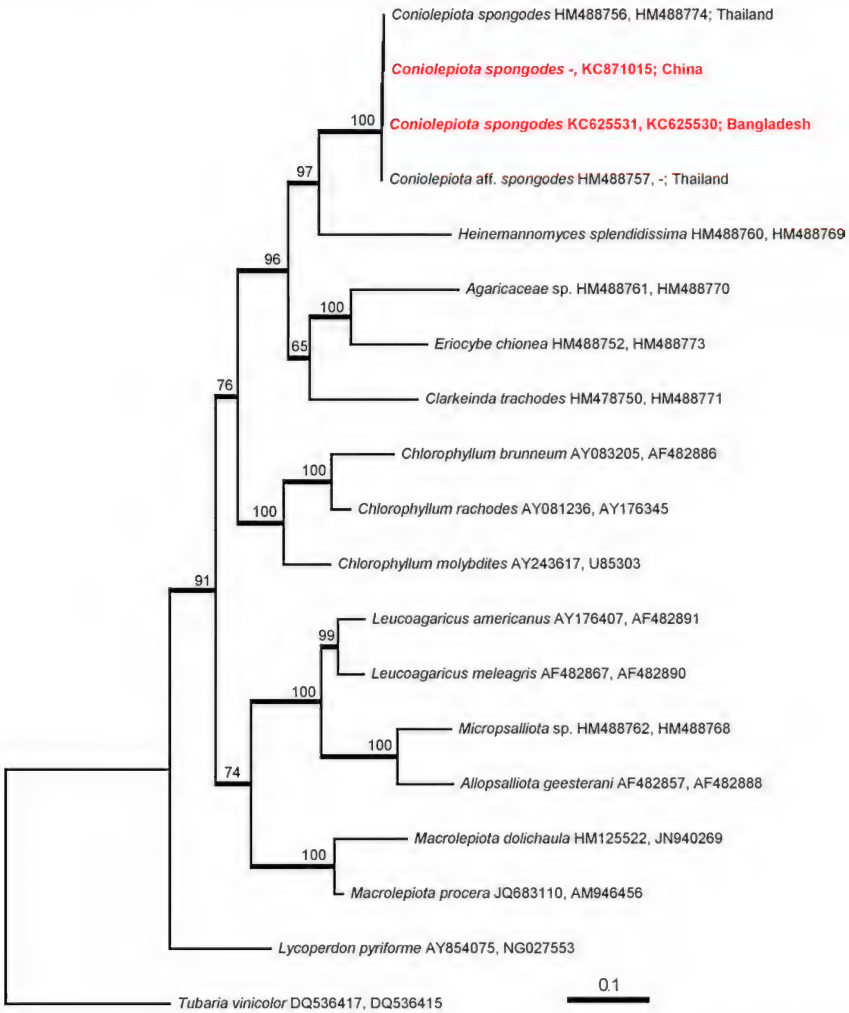

FIGURE 1. A phylogenetic tree generated from the combined ITS–nrLSU dataset of *Agaricaceae* using maximum likelihood (ML) method. Posterior probabilities from Bayesian inference (BI) (>0.97) are indicated as thick branches and bootstrap values derived from ML (BS>60%) are shown above the branches at nodes. Newly generated sequences for *Coniolepiota spongodes* are highlighted in boldface with red color. GenBank accessions ITS and nrLSU are provided after the species name and unavailable sequence is marked as hyphen sign.

(a–)b–c(–d); in which 'b–c' contains at least 90% of the measured values and extreme values 'a' and 'd' are set in parentheses. $Q_m = Q \pm SD$; Q refers to the ratio of individual spore, Q_m refers to the average Q of basidiospores, and SD is the standard deviation.

Molecular procedures and phylogenetic analysis

The protocol for DNA extraction followed those of Doyle & Doyle (1987). ITS1/ITS4 (White et al. 1090) and LROR/LR5 (Vilgalys & Hester 1990) primer pairs were used for the amplification of internal transcribed spacer (ITS) and the large subunit nuclear ribosomal RNA (nrLSU) regions, respectively. PCR amplification, sequencing and sequence alignment, Maximum Likelihood (ML) and Bayesian Inference (BI) methods followed those in Hosen et al. (2013). Phylogenetic analysis was conducted on the combined dataset of the ITS and the nrLSU.

Molecular results

Two sequences were newly generated in this study and are deposited in GenBank. The combined ITS-nrLSU dataset consisted of 1689 nucleotide sites (including gaps). In this dataset, 1116 characters were constant, while 573 characters were variable, of which 211 were parsimony uninformative and 362 were parsimony informative. Parsimony analysis resulted in one most parsimonious tree of 1329 steps, with Consistency Index (CI) = 0.634, Retention Index (RI) = 0.590 and Rescaled Consistency Index (RC) = 0.374. Tree topologies obtained from both ML and BI methods of phylogenetic analysis were almost identical. Sequences from collection of *C. spongodes* made from Bangladesh fall in a highly supported clade with those described from northern Thailand (Fig. 1).

Taxonomy

Coniolepiota spongodes (Berk. & Broome) Vellinga, Mycologia 103: 502, 2011.

Figs 2, 3

Basidiomata solitary, medium-sized. Pileus 40–80 mm broad, plano-convex to applanate with slightly inflexed margin, covered with lilac to purple-gray powdery velar warts at young stage which remain in some areas especially in the center, but in outer zone sometimes disappearing, showing the pallid to whitish background; warts thick at center, becoming spread out towards margin, sometimes with powdery warts hanging over the margin. Lamellae 14 × 4 mm, free and remote from the stipe, crowded with lamellulae, pallid cream to pale yellowish. Stipe 25–50 × 6–10 mm, central, cylindrical, usually slightly tapering upward, glabrous and pallid white at apex but the remaining parts covered with powdery lilac warts as on pileus, base slightly swollen and covered with whitish mycelium, hollow. Annulus present, thin membranous and easily detachable. Context 7 mm thick at the center of the pileus, dull white to slightly pale purplish.

FIGURE 2. *Coniolepiota spongodes*. a–b. Basidiomata show gray lilac velar coverings on the surface of pileus and stipe. c. Hymenophore showing free, crowded, pallid to cream-white lamellae with powdery warts hanging over the margin. d. Gray lilac velar coverings disappearing from the surface of the pileus and stipe at maturity and/or handing. (a = HKAS 77576; b = HKAS 60246; c = SHAF 5; d = HKAS 77574). Photos: a, c, d, Md. Iqbal Hosen; b, Zai-Wei Ge.

BASIDIOSPORES [80/4/4] 4–5(–6) × (2.8–)3–3.2(–3.5) µm [Q = 1.43–1.57, Q_m = 1.52 ± 0.127], ellipsoid to somewhat oblong, hyaline to pale yellowish, dextrinoid, inamyloid, nearly rounded apex, smooth, slightly thick-walled, without germ pore. BASIDIA 12–20 × 5–7 µm, cylindro-clavate to clavate, hyaline to pale yellowish in H_2O, thin-walled, tetrasporic but occasionally 2- or 3-spored. CHEILOCYSTIDIA 20–26 × 6–9 µm, clavate to clavulate, some cylindro-clavate, hyaline, thin-walled, smooth, without encrustations, not abundant, scattered and sometimes hard to find. PLEUROCYSTIDIA not observed. PILEUS COVERING a powdery layer made up of irregularly shaped cylindrical cells, three dimensional (T-shaped), some anastomosing (H-shaped), individual elements 20–50 × 4–10 µm, hyaline, smooth, without incrusting pigments, refractive connecting points often present at the septate portion or near to the ending points of the pileal hyphae. STIPE COVERING similar to those of pileus but refractive connecting points is less common. CLAMP CONNECTIONS absent in all tissues.

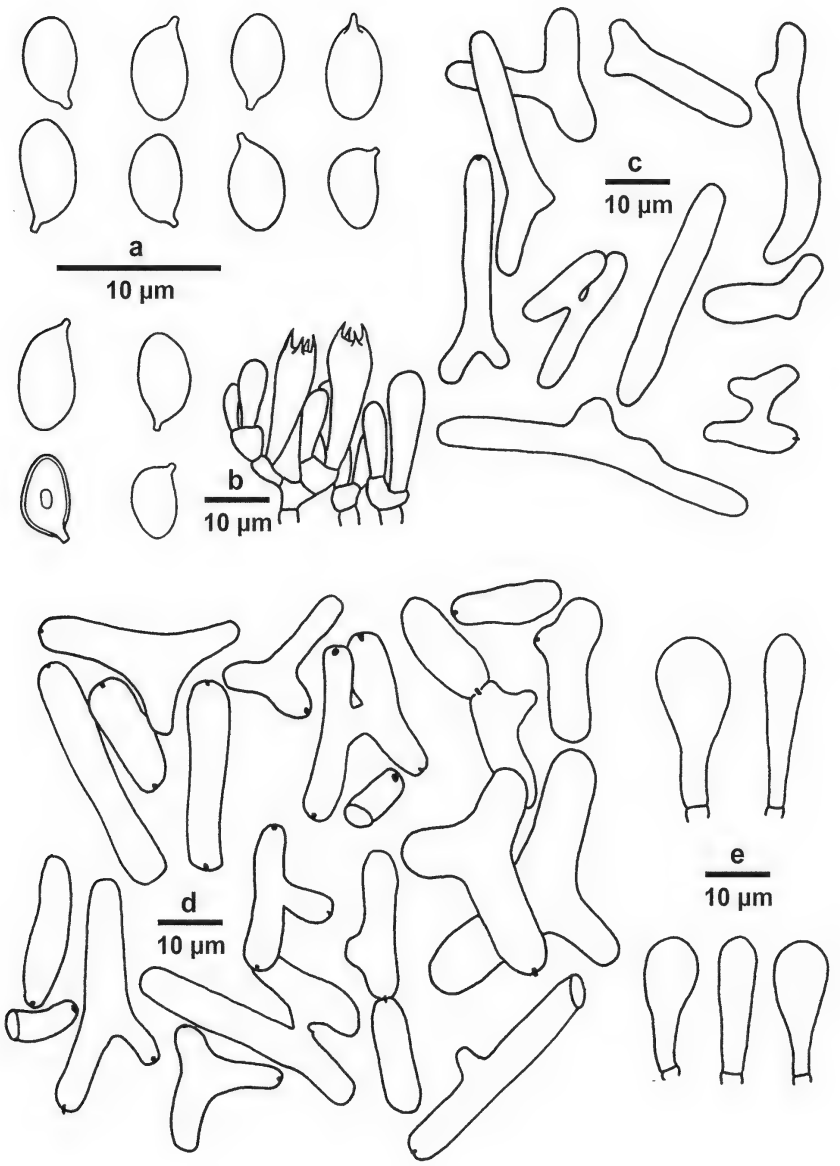

FIGURE 3. *Coniolepiota spongodes*. a. Basidiospores. b. Basidia at different stages of development; c. Stipe covering elements; d. Pileus covering elements; e. Cheilocystidia. (a = HKAS 77575; b–e = HKAS 77576).

Habit & Distribution — Solitary or in small groups of two along roadsides in forests of *S. robusta*, in Bangladesh. Known also from Sri Lanka, Thailand, Singapore, Malaysia, tropical China.

Specimens examined — **BANGLADESH. Rangpur Division**: Thakurgaon, Pirganj, Thumnia Sal Baghan, 70 m a.s.l., 18 July 2011, M.I. Hosen 239a (HKAS 77574; GenBank KC625530, KC625531); 18 June 2012, M.I. Hosen 373 (HKAS 77575); Dinajpur, Birganj, Singra Forest, 60 m a.s.l., 1 July 2013, M.I. Hosen 708 (SHAF 5); **Dhaka Division**: Tangail, Madhupur, Madupur National Park, 20 m a.s.l, 3 August 2012, M.I. Hosen 561 (HKAS 77576). **CHINA. Yunnan Province**: Luxi, Santai Mountain, 1200 m a.s.l., 29 July 1979, Zheng Wen Kang 79078 (HKAS 4848); Menhai, Mangao Nature Reserve, 1250 m a.s.l., 16 August 1991, Zhu L. Yang 1590 (HKAS 24071); Ruili, Nongdao, 1130 m a.s.l., 31 July 2003, Luo Hong 92 (HKAS 43631); **Hainan Province**: Wuzhishan, Wuzhishan Nature Reserve, 1867 m a.s.l., 1 August 2010, Z.W. Ge 2570 (HKAS 60246; GenBank KC871015).

Comments — *Coniolepiota spongodes* is restricted to southeast and south Asia. It is an attractive species for mushroom pickers as it can be readily recognized by the purplish to gray lilac coverings of the pileus and stipe surface. Distinguishing microscopic characters include the absence of abundant cheilocystidia, small smooth ellipsoid to oblong basidiospores, cylindrical elements in the pileal and stipe coverings, and absence of clamp connections in all tissues. Vellinga, who established *Coniolepiota* as an independent genus in *Agaricaceae* based on morphological and molecular evidence, presented the full synonymy of *C. spongodes* (Vellinga et al. 2011). According to Pegler (1972, 1986) basidiospores of *C. spongodes* are dextrinoid, which was confirmed by our study. The Chinese materials of *C. spongodes* also bear the same features that were observed in Bangladesh specimens: pileus and stipe coverings gray purple, basidiospores measuring [4/4/80] 4–5.5 × (2.5–)3–3.5(–4.0) µm [Q = 1.41–1.67, Q_m = 1.52 ± 0.118], ellipsoid, colorless, dextrinoid, inamyloid, slightly thick-walled, and without germ pore. Vellinga et al. (2011) reported that the basidiospores from Thai collections are pale or barely weakly dextrinoid in Melzer's reagent.

Phylogenetically, *C. spongodes* nests within the *Agaricus* s.l. clade based on multi-gene analysis with four different loci (ITS, nrLSU, tef1-α, and rpb2; Vellinga et al. 2011). *Coniolepiota spongodes* is closely related to the colored-spored genera *Agaricus* L., *Heinemannomyces* Watling, and *Clarkeinda* Kuntze (Vellinga et al. 2011). In our molecular analysis *H. splendidissima* Watling is strongly supported to be sister group to *Coniolepiota* with ML (96%) and BI (1.0) values, followed by *Agaricaceae* sp., *Eriocybe chionea* Vellinga and *Clarkeinda trachodes* (Berk.) Singer (Fig. 1). Vellinga et al. (2011) described the genus *Coniolepiota* to include collections of *C. spongodes* from Malaysia, Singapore, and Thailand. A collection from Thailand (ECV 3613), described as having pink-tinges to the pileus and stipe, was designated as *C.* aff. *spongodes* but not formally named. Minor differences in cheilocystidia shape and color of basidiomata were possibly due to environmental variation. Inclusion of our

Bangladesh and Chinese collections in phylogenetic analysis of ITS-nrLSU support the recognition of a single species in the genus rather than two distinct, sister species.

Acknowledgments

This study was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-EW-Z-9). Dr. Zai-Wei Ge (China) is gratefully acknowledged for providing photo of *C. spongodes*. The authors are grateful and sincere thanks to Dr. Teresa Lebel (Australia) and Dr. Takamichi Orihara (Japan) for their critical revision, comments, and reviewing this manuscript prior to submission.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.349

Volume 124, pp. 349–352 *April–June 2013*

Pertusaria albiglobosa, a new lichen from China

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Abstract — *Pertusaria albiglobosa* is described as new, based on collections from Shaanxi province in China. The species is characterized by the white thallus, spherical verrucae, asci with 2 smooth-walled ascospores, and the presence of 2′-O-methylperlatolic acid and 4,5-dichlorolichexanthone. It grows on rotten mosses in alpine meadow at elevations of 3570–3760 m.

Key words — *Pertusariaceae*, *Pertusariales*, lichenized fungi, western China

Introduction

Species of *Pertusaria* DC. colonize almost all habitats. The apothecial structure, the number and structure of the ascospores and the chemistry are the diagnostic characters in identification of *Pertusaria* species (Oshio 1968, Dibben 1980, Archer 1997, Schmitt & Lumbsch 2004). In China, the genus received little attention until Zhao et al. (2004) published an annotated key to the revised taxa of *Pertusaria* from China in which 41 species and 4 varieties were accepted. A recent study of the *Pertusaria* specimens collected from Qinling Mountains in western China revealed a new species, described here.

Materials & methods

All specimens examined are preserved in SDNU (the Lichen Section of Botanical Herbarium, Shandong Normal University). An Olympus SZ 51stereomicroscope and Olympus CX 21 compound microscope were routinely used for the morphological and anatomical examinations. Colour reactions (spot tests) were made using standard methods (Orange et al. 2001). The chemical constituents were identified using thin layer chromatography (TLC) (Culberson 1972) and gradient-elution high performance liquid chromatography (HPLC) (Lumbsch 2002).

Pertusaria albiglobosa Q. Ren, **sp. nov.** Plate 1

MycoBank MB 804971

Differs from *Pertusaria trochiscea* by its 2-spored asci, its production of 2′-O-methylperlatolic acid, and its lack of stictic acid.

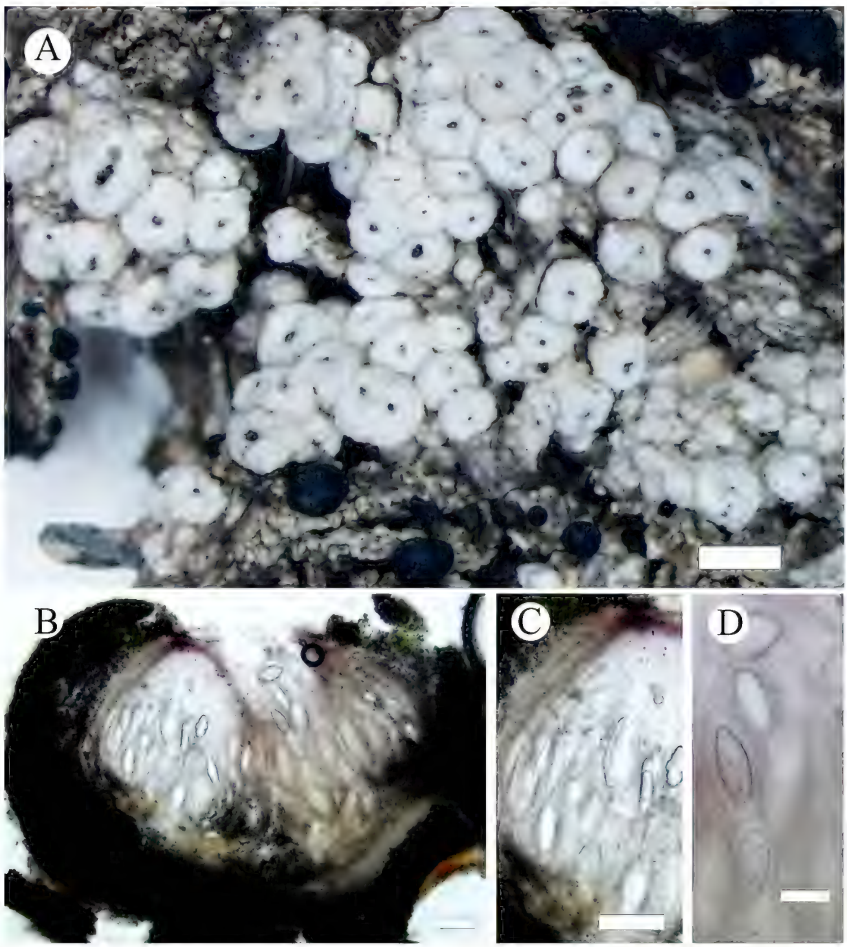

PLATE 1. *Pertusaria albiglobosa* (J.Zh. Zhao 899, SDNU). A. Thallus; B. Apothecium, cross-section; C–D. Asci with 2 ascospores. Bars: A = 1 mm; B & C = 100 µm; D = 50 µm.

TYPE: China. Shaanxi: Taibai Co., Mt. Qinling, Baxiantai, 33°67′N 107°58′E, alt. 3760 m, 5 Aug. 2005, J.Zh. Zhao 899 (**Holotype,** SDNU).

ETYMOLOGY: Latin *albiglobosa*, referring to the colour of the thallus and the shape of the fertile verrucae.

THALLUS white or whitish-gray, episubstratal, thin or fairly thick, the margin entire and unzoned; SURFACE tuberculate, matt and continuous; ISIDIA AND SOREDIA absent. VERRUCAE numerous, crowded or well dispersed, but occasionally fused, (0.5–)0.7–1.0(–1.2) mm in diameter, concolorous with the thallus, persistently closed and appearing as a spherical protuberance;

OSTIOLES single, black. APOTHECIA 1 (occasionally 2–3) per verruca, the fruit center hyaline; EPITHECIUM brown, K+ violet, hypothecium hyaline, hymenium colorless, 325–450 µm thick. ASCI clavate or cylindric; ASCOSPORES 2 per ascus, uniseriate, ellipsoid to fusiform (at times deformed), 63–135(–163) × 30–55 µm, the spore wall double, smooth, 7.5–10 µm thick, and usually trimmed, the end wall 12.5–17.5 µm thick. PYCNIDIA unknown.

SPOT TESTS — cortex: all chemical tests negative, UV–; medulla: all chemical tests negative.

SECONDARY METABOLITES — 4,5-dichlorolichexanthone and 2′-O-methylperlatolic acid (HPLC).

ECOLOGY — *Pertusaria albiglobosa* is a muscicolous species occurring in alpine meadows. It is known only from Shaanxi province (western China) at elevations of 3570–3760 m.

ADDITIONAL SPECIMENS EXAMINED — **CHINA. SHAANXI**: Taibai Co., Mt. Qinling, Wengong Temple, alt. 3570 m, 5 Aug. 2005, Ch.L Wang & F. Yang TBW052 (SDNU); alt. 3600 m, 5 Aug. 2005, Sh.X. Guo & Y.J. Li QL004 (SDNU); Baxiantai, alt. 3767 m, 5 Aug. 2005, Sh.X. Guo & X.L. Shi QL191 (SDNU).

COMMENTS — *Pertusaria albiglobosa* is characterised by the matte white thallus, spherical verrucae, 2-spored asci, smooth-walled spores, and chemistry. In morphology and habitat, this species is very similar to *P. trochiscea* Norman and *Megaspora verrucosa* (Ach.) L. Arcadia & A. Nordin, which differ in number of ascospores per ascus and the chemistry: *P. trochiscea* produces 4 spores per ascus and 4,5-dichlorolichexanthone and stictic acid, and *M. verrucosa* produces 8 spores per ascus and no lichen products. *Pertusaria saximontana* Wetmore also has a similar anatomy and chemistry but is readily distinguished by the shiny gray thallus, wide blackish discs usually surrounded by an eroded inner verrucal margin, smaller ascospores, and its habitats. *Pertusaria signyae* Øvstedal, which might be confused with the new species, is also a muscicolous (also saxicolous) species with 2 smooth spores per ascus, but it has isidia and contains 2'-O-methylperlatolic acid only.

Acknowledgements

The author is grateful to Prof. Jack Elix (Australian National University) for his help in confirming the chemistry and to Dr. Alan W. Archer (Royal Botanic Gardens, Australia) for professional advice. The author thanks Drs. André Aptroot (ABL Herbarium, Soest, The Netherlands) and Shouyu Guo (Institute of Microbiology, CAS) for presubmission review. This study was supported by the National Natural Science Foundation (nos. 31100011 & 31093440).

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.353

Volume 124, pp. 353–359 *April–June 2013*

Lichenological notes 6: nomenclatural acts

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Abstract — *Lecanora peltastictoides* is transferred to *Aspicilia*. *Thelenella americana* is treated as *Melanophloea americana* and *T. montana* as *M. montana*. *Sarcogyne athroocarpa* is treated as a synonym of *Acarospora badiofusca* and a lectotype is designated. *Pleopsidium stenosporum* is treated as a synonym of *P. flavum*.

Key words — *Acarosporaceae*, Joshua Tree National Park, *Thelocarpaceae*

The species

Acarospora badiofusca (Nyl.) Th. Fr., Nova Acta Regiae Soc. Sci. Upsal., Ser. 3: 3 190 (1861).

≡ *Lecanora badiofusca* Nyl., Herb. Mus. Fenn.: 110 (1859).

Type: Ad saxa granitica in Lapponia, Nylander s.n. (H-NYL! holotype).

= *Sarcogyne athroocarpa* H. Magn., Ann. Crypt. Exot. 7: 137 (1935) ["1934"], **syn. nov.**

Type: U.S.A., Colorado, Boulder Co., Sugar Loaf, 2743 m, on granite, Aug. 1898, Morgan s.n., Lichenes Boreali-Americani 227 (FH! holotype, destroyed by sampling?; COLO! L-79776, **lectotype designated here**).

The holotype collection of *Sarcogyne athroocarpa* has only a *Lecidea* species remaining, and the *Sarcogyne* is presumed to have been destroyed by sampling. In COLO we have discovered a syntype of *S. athroocarpa*, originally identified by W.A. Weber, which we designate as a lectotype. Unfamiliar with extremely reduced forms of *A. badiofusca* in North America at high elevations, H. Magnusson, who described the reduced dispersed apothecia of *A. badiofusca* as an endolithic *Sarcogyne*, suggested that its sterile areoles represented another "*Acarospora*" species (Magnusson 1935). As with *A. badiofusca*, the hymenium is low (60–70 μm) and the perithecial crown (exciple) is brown (it often becomes

blackened in many specimens). Therefore, we place *Sarcogyne athroocarpa* in synonymy with *A. badiofusca.*

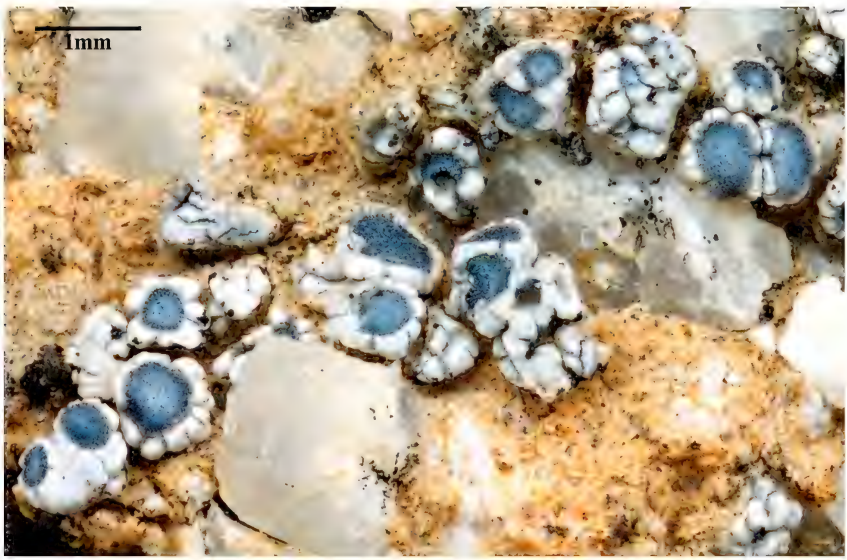

Plate 1. *Aspicilia peltastictoides* (Knudsen et al. 14420, UCR), Coxcomb Mountains, Joshua Tree National Park. (Tim Wheeler, photo). Scale bar = 1 mm.

Aspicilia peltastictoides (Hasse) K. Knudsen & Kocourk., **comb. nov.** Plate 1

MycoBank MB 803946

≡ *Lecanora peltastictoides* Hasse, Bryologist 17: 63 (1914).

Type: U.S.A., California, Palm Springs, on granite, 1901, Hasse 861 (FH!, holotype)

In 1914, the pioneer California lichenologist H.E. Hasse (1836–1915) described *Lecanora peltastictoides* from granite in the Sonoran Desert in Palm Springs, California. During the revision of *Lecanora* for the Sonoran Lichen Flora, no specimens besides the type were seen, and the species remained unrevised but recognized as an *Aspicilia* (Knudsen 2003; Ryan et al. 2004). During our inventory of Joshua Tree National Park, we collected five specimens of *Lecanora peltastictoides* in the Mojave and Sonoran Deserts.

Lecanora peltastictoides is an *Aspicilia* with small ascospores (mostly 12–13 × 9–10 µm), eight per ascus, a white striated thallus, black pruinose apothecia with elevated crenulate margins, and no secondary metabolites. No conidia have been observed. It occurs on granite, usually on softer decaying rock at Joshua Tree National Park. There are only two other *Aspicilia* species in California with similar sized ascospores. Both species, *A. brucei* Owe.-Larss. & A. Nordin and *A. cinerea* (L.) Körb., can easily be distinguished from *A. peltastictoides*

by their production of norstictic acid. *Aspicilia peltastictoides* looks similar to two other lichen species in southern California, *Acarospora strigata* (Nyl.) Jatta and *Lecanora utahensis* H. Magn. The polyspored asci easily distinguish *A. strigata* from *A. peltastictoides*. An older name for *A. strigata* in North America is *A. peltasticta* Zahlbr., which explains the etymology of the specific name of *peltastictoides*. *Lecanora utahensis* has been confused with *A. peltastictoides* not only because they look similar, but because they both have similar sized ascospores (Knudsen & Lendemer 2006; Knudsen 2012). *Lecanora utahensis*, a member of the *Lecanora dispersa* group (Śliwa 2007), differs especially in the production of isousnic acid and the *Lecanora*-type ascus stain. We regard both *A. peltastictoides* and *L. utahensis* as rare in California.

Additional specimens examined — U.S.A., California, Riverside Co., Little San Bernardino Mountains, Berdoo Canyon, 1025 m, rare on granite along wash, 7 Dec. 2010, Knudsen 12869.2 & Harding (UCR); upper Covington Flats, 1426 m, on granite, 9 Nov. 2011, Knudsen 14043 & Kocourková (UCR); San Bernardino Co., 49 Palms Canyon, 844 m, on granite boulder in palm oasis, 29 Dec. 2010, Knudsen 13469 (UCR); Coxscomb Mountains, 635 m, on decaying granite boulder, 7 Dec. 2011, Knudsen et al. 14420 (UCR).

Melanophloea americana K. Knudsen & Lendemer, Opusc. Philolich. 9: 61 (2011).

≡ *Thelenella americana* (K. Knudsen & Lendemer) Aptroot, Lichenologist 44: 504 (2012).

Type: U.S.A., Pennsylvania, Montgomery Co., Pennypack Watershed, on silicate rock, Nov. 1987, A. Aptroot 21376 (NY! holotype; ABL[n.v.], BR[n.v.], isotypes).

Melanophloea montana P.M. McCarthy, Australas. Lichenol. 62: 26 (2008).

≡ *Thelenella montana* (P.M. McCarthy) Aptroot, Lichenologist 44: 506 (2012).

Type: Australia, Queensland, Atherton Tableland, Tully Falls, on siliceous rock, 7 August 2006, McCarthy 2520 (CANB, holotype, n.v.).

Melanophloea (*Thelocarpaceae*) contains three species. The type of the genus is *M. pacifica* P. James & Vězda, described from the Solomon Islands (James & Vězda 1971). The saxicolous *M. montana* was described from Australia (McCarthy 2008), and the saxicolous *M. americana* was described from eastern North America (Knudsen et al. 2011). Recently Aptroot & Schumm (2012) transferred *M. americana* and *M. montana* from the *Thelocarpaceae* to *Thelenella* in the *Thelenellaceae*. *Melanophloea americana* and *M. montana* differ from *Thelenella* species in the following important characters that were overlooked by these authors:

1. *Melanophloea americana* and *M. montana* have *Thelocarpon*-like ascomata that are apothecioid with an inner wall that is an incurving exciple, reducing the size of the hymenial disc (clearly visible when wetted), and an outer wall that is basically a thalline margin (Salisbury 1966; Horáková 1998; Knudsen & Lumbsch 2007; McCarthy 2008; Knudsen et al. 2011). In *M. americana* and

M. pacifica, algae are scattered between the outer and inner walls, extending up from the algal layer in the thallus. This apothecioid fruiting body differs fundamentally from the single-walled perithecia of *Thelenella* species (Mayrhofer & Poelt 1985; Mayrhofer 2002; Orange et al. 2009).

2. *Melanophloea americana* and *M. montana* differ from *Thelenella* species in having apothecioid ascomata with distinct outer and inner walls. The outer or thalline wall of the ascomata in the *Melanophloea* species is melanized, with the melanization of the outer thalline wall possibly protecting the algae beneath. The inner wall (an incurving exciple) is hyaline. Aptroot & Schumm (2012) misinterpreted the outer wall of *M. americana* as a clypeus, which we define as a wall in corticolous species formed of fungal and bark cells, not the melanized wall of a saxicolous fungus containing no distinct substrate particles and which is basically thalline (Nash et al. 2002). *Thelenella* has single-walled perithecia, also without a clypeus using the definition we accept (Mayrhofer & Poelt 1985; Mayrhofer 2002; Orange et al. 2009).

3. *Melanophloea americana* and *M. montana* have hyaline simple ascospores in polyspored asci with a K/I+ blue stain, while all *Thelenella* taxa have 2–8-spored asci with a K/I– stain and large muriform or submuriform spores (Mayrhofer & Poelt 1985; Mayrhofer 2002; Orange et al. 2009).

Based on these characters we reject the emendation of *Thelenellaceae* proposed by Aptroot & Schumm (2012). The speculations of Aptroot & Schumm (2012) about the evolutionary development of polyspory are not relevant in this case because *M. americana* and *M. montana* are not *Thelenella* species.

The radical emendation of *Thelenella* by Aptroot & Schumm (2012) lacks not only convincing morphological evidence but molecular support. While we are open to the possibility that *M. americana* and *M. montana* are not congeneric with *M. pacifica*, to radically emend another genus and family lacking molecular data and strong morphological support is speculative and undermines nomenclatural stability. That molecular data are required to confirm their hypothesis is reinforced by the fact that *M. americana* and *M. montana* differ from *Thelenella* in significant morphological characters.

Until these further results are obtained, we treat *T. americana* and *T. montana* as members of *Melanophloea* using the names *M. americana* and *M. montana*.

It should be noted that Aptroot & Schumm (2012) cited inaccurate type collections for *Melanophloea americana*. When the name *M. americana* was introduced, the type collection was clearly stated to be Aptroot 21376 (with the holotype deposited at NY). A second collection from the same site as the holotype, Aptroot 21389, was used by NY curator R.C. Harris to produce the microscopic images for the protologue to preserve the holotype material. Although Aptroot 21389 was cited in the figure caption in the protologue, it was inadvertently omitted from list of additional specimens examined. This

collection (Aptroot 21389) is a paratype or a topotype of *M. americana*, but it is not an isotype as stated by Aptroot & Schumm (2012: 504), because a separate collection number clearly indicates it does not represent part of the same gathering as the type. The holotype of *M. americana* consists of a large rock, with abundant and well developed thalli and ascomata. Although the quality of a specimen is irrelevant from a nomenclatural standpoint, this particular collection was selected as the type because of its exemplary condition compared with the other available material.

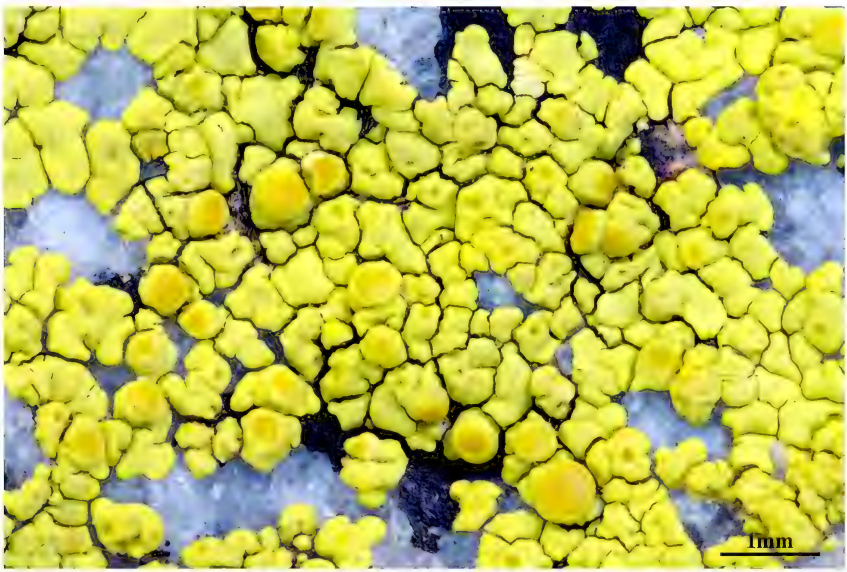

Plate 2. *Pleopsidium flavum* (Knudsen 12911, UCR), Joshua Tree National Park. (Tim Wheeler, photo). Scale bar = 1 mm.

Pleopsidium flavum Körb., Syst. Lich. Germ.: 113 (1855). Plate 2

Type: Switzerland, Schaerer, Lich. Helvet. exs. No. 335, sub *Parmelia flava* a *oxytona* (GZU, neotype, designated by Hafellner 1993, n.v.).

= *Lecanora stenospora* Stizenb. ex Hasse, Bull. Torrey Bot. Club 24: 447. 1897, **syn. nov.**

≡ *Acarospora stenospora* (Stizenb. ex Hasse) Hue, Nouv. Arch. Mus. Hist. Nat., Sér. 5, 1: 161 (1909).

≡ *Acarospora stenospora* (Stizenb. ex Hasse) Zahlbr., Cat. Lich. Univ. 5: 108 (1928), nom. illeg. superf.

≡ *Pleopsidium stenosporum* (Stizenb. ex Hasse) K. Knudsen, Opusc. Philolich. 9: 79 (2011).

Type: U.S.A., California, [Los Angeles or San Bernardino Co.] San Gabriel Mountains, above 800 m, on unknown rock type with some HCl+ inclusions, 1895, Hasse s.n. (NY! [Hasse Herb. No. 3682], neotype designated Knudsen 2011).

Lecanora stenospora was described from the San Gabriel Mountains in southern California. The holotype was missing from ZT and a neotype from NY was designated. The name was transferred to *Pleopsidium* based on its distinctive *Pleopsidium*-type ascus stain (Knudsen 2011). Due to the dispersed thallus, wandering lines of areoles, and a possible difference in the production of secondary metabolites, *P. stenosporum* was tentatively treated as a separate species from *P. flavum*. Field studies in Joshua Tree National Park and Yosemite National Park by the first author, as well as in Montana by Tim Wheeler, convinced us that the normally contiguous and effigurate thallus of *P. flavum* will become dispersed or form long lines of areoles, especially when growing in shaded overhangs or if infected with *Lichenostigma saxicola* K. Knudsen & Kocourk. Michalová (2012), who investigated the neotype for secondary metabolites, detected acaranoic acid, a substance typically present with rhizocarpic acid in *P. flavum*. Since *P. stenosporum* does not differ from *P. flavum* in thallus morphology or secondary metabolites, it is treated as a synonym of *P. flavum,* which is common in North America.

Acknowledgments

We thank our reviewers, J.C. Lendemer (NY) and P.M. McCarthy (Australia). The work of Kerry Knudsen was supported by a co-operative agreement between Joshua Tree National Park and the University of California at Riverside. The work of Jana Kocourková was supported financially by the Kontakt II, Program of International Cooperation in Research and Development for scientific cooperation between the CR and USA, LH 11057 from Ministry of Education, Youth and Sports. We thank for their help the curators of COLO, FH, and ZT. We thank Tim Wheeler (Montana) for discussion about *Pleopsidium* and for the figures.

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ISSN (print) 0093-4666 © 2013. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.361

Volume 124, pp. 361–365 *April–June 2013*

Phylloporia tiliae sp. nov. from China

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Abstract — A new species, *Phylloporia tiliae*, is described and illustrated from Hunan Province, China. It is distinguished from other *Phylloporia* species by its combination of a perennial habit, pileate basidiocarps, a crusted pileal surface with tomentose margin, minute pores, and a monomitic hyphal system.

Key words — *Hymenochaetaceae, Hymenochaetales*, polypore, taxonomy

Introduction

Phylloporia Murrill was erected for *P. parasitica* Murrill, growing on living leaves (Murrill 1904). Most species of this genus have been found on living trees and also sometimes on dead wood (Zhou & Dai 2012). The genus has a worldwide distribution (Ryvarden & Johansen 1980, Gilbertson & Ryvarden 1987, Ryvarden & Gilbertson 1994, Núñez & Ryvarden 2000, Dai 2010, 2012), and includes a total of 24 species (Zhou & Dai 2012, Decock et al. 2013).

Phylloporia is a monophyletic genus within the *Hymenochaetaceae* (Valenzuela et al. 2011, Zhou & Dai 2012). All *Phylloporia* species share abundant thick-walled tiny colored basidiospores and lack setae (Wagner & Ryvarden 2002, Ipulet & Ryvarden 2005, Douanla-Meli et al. 2007, Cui et al. 2010, Valenzuela et al. 2011, Zhou & Dai 2012); however, this genus is highly diverse in morphology and life strategy (see Zhou & Dai 2012 for detail).

Cui et al. (2010) reported two new species of *Phylloporia* from South China. Recently, Zhou & Dai (2012) described five new species from all over China based on morphological and phylogenetic evidence and provided a key to worldwide species thus far accepted in *Phylloporia*. However, there are still a number of Chinese specimens that show generic characters but have not been identified to species level. One of these is described and illustrated here as *Phylloporia tiliae*.

Materials & methods

The holotype specimen was deposited at the herbarium of Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China (IFP). The microscopic procedure follows Zhao et al. (2013). The following abbreviations are used: IKI = Melzer's reagent, IKI– = inamyloid and non-dextrinoid, CB = Cotton Blue, CB(+) = weakly cyanophilous, KOH = 5% potassium hydroxide, L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens, and n = number of basidiospores measured/number of specimens measured. Sections prepared in IKI, CB and KOH solutions were studied using a Nikon Eclipse 80i microscope at magnifications up to ×1000. When presenting the basidiospore size variation, the upper and lower 5% of measurements are excluded from the range and the extreme values are presented in parentheses. Line drawings were made with the aid of a light tube. Special color terms follow Petersen (1996).

Taxonomy

Phylloporia tiliae L.W. Zhou, **sp. nov.** FIGURE 1

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Differs from *Phylloporia pectinata* in its monomitic hyphal system and narrower basidiospores.

TYPE: China. Hunan Province, Zhangjiajie, Tianmenshan National Forest Park, on living *Tilia* (*Malvaceae*), 19.VIII.2010, Yuan 5491 (**holotype**, IFP).

ETYMOLOGY: *tiliae* (Lat.): refers to the host genus.

BASIDIOCARPS perennial, pileate, single, without odor or taste when fresh. PILEI dimidiate, projecting up to 7.5 cm, 5.5 cm wide, and 2 cm thick at base. PILEAL SURFACE glabrous, crust, mouse-grey, azonate; MARGIN tomentose, obtuse, curry-yellow to yellowish brown, a distinct groove between margin and other part of the pileal surface. PORE SURFACE honey-yellow, glancing; STERILE MARGIN distinct, curry-yellow, up to 1 mm wide; PORES circular, 9–12 per mm; DISSEPIMENTS thin, entire. CONTEXT duplex when juvenile, up to 6.5 mm thick, with a black line, the lower context woody hard, yellowish brown, up to 2.5 mm thick, upper context as a tomentum, soft corky, cinnamon-buff, yellowish brown, up to 4 mm thick, the black line becoming a crust with age. TUBES yellowish brown, woody, each layer up to 1 mm long.

HYPHAL SYSTEM monomitic; GENERATIVE HYPHAE simple septate; tissue becoming reddish brown in KOH but otherwise unchanged. CONTEXTUAL HYPHAE pale yellowish to yellowish, slightly thick- to thick-walled with a wide lumen, rarely branched, frequently simple septate, straight, regularly arranged, 2.5–4.5 μm in diam; HYPHAE IN TOMENTUM yellowish brown, slightly thick-walled, occasionally branched, frequently simple septate, loosely interwoven, 2–5 μm in diam; HYPHAE IN THE BLACK ZONE (CRUST) dark brown, distinctly thick-walled with a narrow lumen, strongly agglutinate, interwoven. TRAMAL

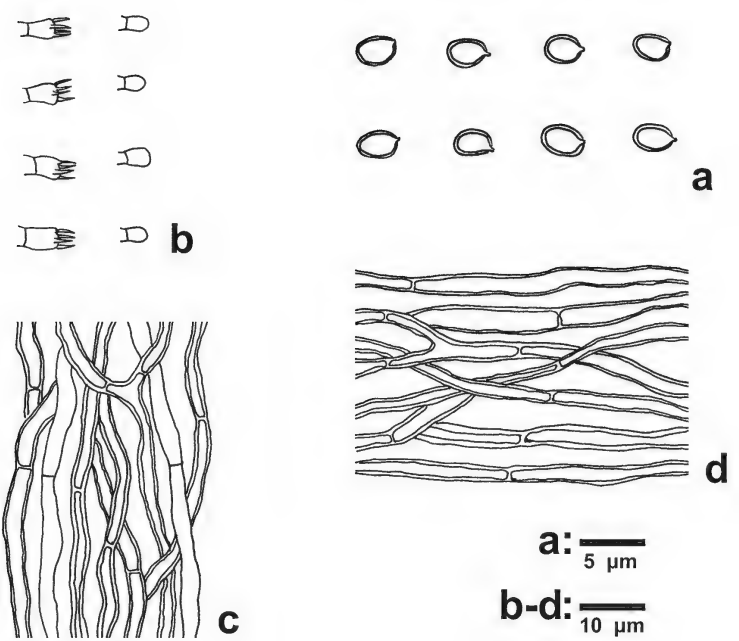

FIGURE 1. *Phylloporia tiliae* (holotype).
a: Basidiospores. b: Basidia and basidioles.
c: Hyphae from trama. d: Hyphae from the lower context.

HYPHAE hyaline, thin-walled to yellowish, thick-walled with a wide to narrow lumen, rarely branched, frequently simple septate, more or less straight, subparallel along the tubes, 2–4 µm in diam. SETAE absent; CYSTIDIA absent; CYSTIDIOLES absent. BASIDIA barrel-shaped with four sterigmata and a simple septum at the base, 4–6 × 3–4.5 µm; BASIDIOLES dominant, similar to basidia in shape, but slightly smaller. BASIDIOSPORES ellipsoid, yellowish, thick-walled, smooth, IKI–, CB(+), (2.8–)3–3.4(–3.5) × (1.9–)2–2.5(–2.6) µm, L = 3.17 µm, W = 2.29 µm, Q = 1.38 (n = 30/1).

REMARKS: *Phylloporia tiliae* has thick-walled tiny colored basidiospores and lacks setae, fulfilling the morphological characters of *Phylloporia*. The new species is characterized by the combination of a perennial habit, pileate basidiocarps, a crusted pileal surface with tomentose margin, small pores, and a monomitic hyphal system.

Macroscopically, *Phylloporia crataegi* L.W. Zhou & Y.C. Dai, *P. ephedrae* (Woron.) Parmasto, *P. gutta* L.W. Zhou & Y.C. Dai, *P. pectinata* (Klotzsch)

Ryvarden, and *P. ribis* (Schumach.) Ryvarden share perennial pileate basidiocarps with *P. tiliae*. However, *P. crataegi, P. ephedrae, P. gutta*, and *P. ribis* have larger pores (<9 per mm; Wagner & Ryvarden 2002, Zhou & Dai 2012).

Phylloporia pectinata has pores (8–10 per mm) similar in size to those of *P. tiliae*, and both species also share a black crust at the old part of pileal surface and duplex context; however, *P. pectinata* has a zonate and sulcate pileal surface with acute margin and (more importantly) a dimitic hyphal system and wider basidiospores (3 μm wide; Wagner & Ryvarden 2002).

Phylloporia fontanesiae L.W. Zhou & Y.C. Dai also has small pores (10–12 per mm) and a monomitic hyphal system like *P. tiliae,* but its annual habit, zonate sulcate pileal surface with acute margin, and shorter basidiospores (2.6–3 μm long; Zhou & Dai 2012) distinguish it from *P. tiliae*. In addition, *P. fontanesiae* was found on living *Fontanesia* (*Oleaceae*) in temperate China in Henan (Zhou & Dai 2012) and Shandong (data unpublished) provinces.

Phellinus minisporus B.K. Cui & Y.C. Dai, which was described from southern China and has similar pores, differs from *Phylloporia tiliae* in its resupinate habit, abundant hymenial setae, and smaller basidiospores (Cui et al. 2009).

Some *Phylloporia* species often inhabit living trees, affecting leaves, twigs, branches, and trunk bases, and have an exclusive host-specificity (Zhou & Dai 2012). This phenomenon exists in certain species of *Inonotus linteus* complex as well (Tian et al. 2003). Therefore, it is important to record the host information when collecting specimens of *Phylloporia* and even other genera in *Hymenochaetaceae*.

Acknowledgements

Dr. Deepika Kumari (Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, China) is thanked for improving this manuscript. I express my gratitude to Prof. Yu-Cheng Dai (Beijing Forestry University, China) and Dr. Viacheslav Spirin (Helsinki University, Finland) who reviewed the manuscript before final submission. The research was financed by the National Natural Science Foundation of China (Project No. 31200015).

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ISSN (print) 0093-4666 © 2013 Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/124.367

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